艾特吉
抗人T细胞猪免疫球蛋白
Anti-human T Lymphocyte Porcine Immunoglobulin

再生障碍性贫血强化免疫治疗文献汇编

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抗人 T 细胞猪免疫球蛋白（P-ATG）

武汉中生毓晋生物医药有限责任公司

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再生障碍性贫血诊断与治疗中国指南(2022年版)

中华医学会血液学分会红细胞疾病(贫血)学组
通信作者:邵宗鸿,天津医科大学总医院血液科,天津 300052,Email:shaozonghong@sina.com;张连生,兰州大学第二医院,兰州 730030,Email:zhanglsh@lzu.edu.cn
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Guidelines for the diagnosis and management of aplastic anemia in China (2022)

Red Blood Cell Disease (Anemia) Group, Chinese Society of Hematology, Chinese Medical Association
Corresponding author: Shao Zonghong, Department of Hematology, General Hospital, Tianjin Medical University, Tianjin 300052, China, Email: shaozonghong@sina.com; Zhang Liansheng, Department of Hematology, Lanzhou University Second Hospital, Lanzhou 730030, China, Email: zhanglsh@lzu.edu.cn

中华医学会血液学分会红细胞疾病(贫血)学组在广泛征集专家建议和意见的基础上,结合再生障碍性贫血(AA)最新诊治进展及我国国情对2017年版AA专家共识进行更新,制订《再生障碍性贫血诊断与治疗中国指南(2022年版)》。

一、AA概述及发病机制

AA是一种骨髓造血衰竭(BMF)综合征。其年发病率在我国为0.74/10万,可发生于各年龄组,AA高发年龄分别为15~25岁的青壮年和65~69岁的老年人[1],男、女发病率无明显差异。AA分为先天性及获得性。目前认为T淋巴细胞异常活化、功能亢进造成骨髓损伤在原发性获得性AA发病机制中占主要地位,新近研究显示辅助性T细胞亚群Th1/Th2分化偏移[2]、调节性T细胞(Treg)及NK细胞调节功能不足[3-5]、Th17[6]、树突状细胞(DC细胞)[7]以及巨噬细胞[8]等功能异常甚至某些遗传背景都参与了AA发病。先天性AA较为罕见,主要为范可尼贫血(FA)、先天性角化不良(DKC)、先天性纯红细胞再生障碍(DBA)、Shwachmann-Diamond综合征(SDS)等。绝大多数AA属获得性,本指南主要讨论原发获得性AA。

二、AA的诊断建议

(一)诊断AA的实验室检测项目

1. 推荐检测项目:①血常规检查:白细胞计数及分类、红细胞计数及形态、HGB水平、网织红细胞百分比和绝对值、血小板计数和形态。②不同平面多部位骨髓穿刺:至少包括髂骨和胸骨。骨髓涂片分析:造血细胞增生程度;粒、红、淋巴系细胞形态和各阶段百分比;巨核细胞数目和形态;小粒造血细胞面积;是否有异常细胞等。③骨髓活检:至少取2 cm骨髓组织(髂骨)标本用以评估骨髓增生程度、各系细胞比例、造血组织分布(有无灶性$CD34^+$细胞分布等)情况,以及是否存在骨髓浸润、骨髓纤维化等。④流式细胞术检测骨髓$CD34^+$细胞数量、阵发性睡眠性血红蛋白尿症(PNH)克隆(CD55、CD59、Flaer)。⑤肝、肾、甲状腺功能,病毒学[包括肝炎病毒、EB病毒(EBV)、巨细胞病毒(CMV)、细小病毒B19等]及免疫球蛋白、补体、免疫固定电泳检查。⑥血清铁蛋白、叶酸和维生素B_{12}水平。⑦免疫相关指标检测:T细胞亚群(如$CD4^+$、$CD8^+$、Th1、Th2、Treg等)、细胞因子(如IFN-γ、IL-4、IL-10等)、NK细胞亚群、自身抗体和风湿抗体,大颗粒淋巴细胞白血病相关标志检测。⑧细胞遗传学:常规染色体核型分析、荧光原位杂交[del(7)、del(7q-)、+8、del(5q)、del(20q)等]以及遗传性疾病筛查(儿童或有家族史者推荐做染色体断裂试验),胎儿血红蛋白检测。⑨其他:心电图、腹部超声、超声心动图及其他影像学检查(如胸部X线或CT等)等。

2. 建议检测项目：有条件的医院可开展以下项目：①骨髓造血细胞膜自身抗体检测；②端粒长度及端粒酶活性检测、端粒酶基因突变检测；③二代测序（NGS）检测有无先天性骨髓衰竭性疾病相关基因突变及克隆造血分子标志。

（二）AA诊断标准

1. 血常规检查：全血细胞（包括网织红细胞）减少，淋巴细胞比例增高。至少符合以下三项中两项：HGB < 100 g/L；PLT < 50×10^9/L；中性粒细胞绝对值（ANC）< 1.5×10^9/L。

2. 骨髓穿刺：多部位（不同平面）骨髓增生减低或重度减低；小粒空虚，非造血细胞（淋巴细胞、网状细胞、浆细胞、肥大细胞等）比例增高；巨核细胞明显减少或缺如；红系、粒系细胞均明显减少。

3. 骨髓活检（髂骨）：全切片增生减低，造血组织减少，非造血细胞增多，网硬蛋白不增加，无异常细胞。

4. 除外检查：必须除外先天性（表1）和其他获得性、继发性BMF（表2）。

（三）AA严重程度确定（Camitta标准）

1. 重型AA（SAA）诊断标准：（1）骨髓细胞增生程度 < 正常的25%；如≥正常的25%但 < 50%，则残存的造血细胞应 < 30%。（2）血常规需具备下列三项中的两项：ANC < 0.5×10^9/L；网织红细胞绝对值 < 20×10^9/L；PLT < 20×10^9/L。（3）若 ANC < 0.2×10^9/L，则诊断为极重型AA（VSAA）。

2. 非重型AA（NSAA）诊断标准：未达到SAA。根据是否依赖血制品输注，将NSAA分为输血依赖型（TD-NSAA）和非输血依赖型（NTD-NSAA），TD-NSAA有向SAA转化风险[9]。成分输血指征：HGB≤60 g/L；PLT≤10×10^9/L，或 PLT≤20×10^9/L 伴有明显出血倾向。平均每8周至少1次成分输血且输血依赖持续时间≥4个月者称为TD-NSAA[10]。

（四）AA鉴别诊断

AA应与其他引起全血细胞减少的疾病相鉴别，见表2。AA属于BMF。BMF可以分为先天性和获得性两种，而获得性BMF又分为原发性和继发性。

1. 其他原发性BMF：其他原发性BMF主要包括：（1）源于造血干细胞质量异常的BMF，包括PNH、骨髓增生异常综合征（MDS）和意义未明克隆性血细胞减少（CCUS）等；（2）自身抗体介导的BMF；（3）意义未明的血细胞减少（ICUS），是某特定疾病的前期阶段，可发展为MDS或其他血液病，也可能是尚未认知的疾病。

2. 继发性BMF：造成继发性BMF的因素较多，主要包括：（1）造血系统肿瘤，如毛细胞白血病（HCL）、大颗粒淋巴细胞白血病（LGL）、多发性骨髓瘤（MM）等；（2）其他系统肿瘤浸润骨髓；（3）骨髓纤维化；（4）严重营养性贫血；（5）急性造血功能停滞；（6）肿瘤性疾病因放化疗所致骨髓抑制等。

三、AA的治疗建议

（一）AA的治疗原则

SAA一经确诊应尽早启动本病治疗，研究显示SAA诊断30 d内启动治疗疗效明显优于30 d后启动治疗组[11]。确诊为SAA患者及TD-NSAA的标准疗法：对年龄≤40岁且有HLA相合同胞供者的SAA患者，如无活动性感染和出血，首选HLA相合同胞

表1　与再生障碍性贫血相鉴别的先天性全血细胞减少症

疾病	临床特征
先天性无巨核细胞性血小板减少症	常染色体隐性遗传，TPO受体c-Mpl基因突变所致；血小板减少或全血细胞减少；骨髓衰竭
先天性角化不良症	遗传方式有X连锁遗传、常染色体显性或隐性遗传；皮肤色素异常、口腔白斑、指甲营养不良三联征；全血细胞减少，肺纤维化等
范可尼贫血	大部分属常染色体隐性遗传，少数（FANCB亚型）为X连锁遗传；主要表现为血细胞减少、先天畸形、幼年癌症，染色体断裂试验阳性，易进展为MDS/AML
RUNX1种系突变	血小板减少或全血少，易进展为MDS/AML
GATA-2缺失综合征	反复感染（分枝杆菌、病毒、真菌等），淋巴水肿，疣，肺泡蛋白沉积症，易进展为MDS/AML
SAMD9/9L异常	MIRAGE（骨髓增生异常，感染，生长受限，肾上腺发育不全，生殖异常，肠病），易进展为MDS/AML
重症先天性中性粒细胞减少症	中性粒细胞减少，反复细菌感染
先天性中性粒细胞减少伴胰腺功能不全综合征	常染色体隐性遗传，多数患者有SBDS基因突变，表现为骨髓衰竭，胰腺外分泌功能不全，易进展为MDS/AML

注　MDS：骨髓增生异常综合征；AML：急性髓系白血病

表2 与AA相鉴别的其他获得性或继发全血细胞减少症

疾病或临床表现	鉴别要点
PNH相关(AA/PNH)	依据疾病及PNH向AA转化的阶段不同,患者的临床表现不同。检测外周血红细胞和白细胞表面GPI锚链蛋白可以鉴别
低增生性MDS/AML	低增生性MDS具备如下特点:增生减低,一系或多系病态造血;外周血可见幼稚细胞;骨髓活检可见网状纤维、$CD34^+$细胞增加以及前体细胞异常定位(ALIP)
自身抗体介导的全血细胞减少	包括Evans综合征等。可检测到外周成熟血细胞的自身抗体或骨髓未成熟血细胞的自身抗体,患者可有全血细胞减少并骨髓增生减低,但外周血网织红细胞或中性粒细胞比例往往不低甚或偏高,骨髓红系细胞比例不低且易见"红系造血岛",Th1/Th2降低(Th2细胞比例增高)、$CD5^+$ B细胞比例增高,血清IL-4和IL-10水平增高,对糖皮质激素和(或)大剂量静脉滴注丙种球蛋白、CD20单抗、CTX等治疗反应较好
大颗粒淋巴细胞(LGL)白血病	可表现为全血细胞减少,和(或)脾大及B症状等。流式细胞术检测外周血持续性LGL数量增多,TCR基因重排等检测证实LGL为克隆性增殖
霍奇金淋巴瘤或非霍奇金淋巴瘤	可表现为全血细胞减少、骨髓增生减低、骨髓涂片可见局部淋巴瘤细胞浸润。AA患者淋巴细胞显著增高,但系正常淋巴细胞,可通过免疫分型和基因重排检测与淋巴瘤细胞进行区分。其他如脾肿大等特征也可作为鉴别AA与淋巴瘤的依据
原发性骨髓纤维化	可表现为全血细胞减少,外周血可检测到泪滴样异常红细胞、幼稚粒细胞/幼稚红细胞,脾肿大。骨髓易干抽,骨髓活检可见巨核细胞增生和异型巨核细胞,网状纤维和(或)胶原纤维
分枝杆菌感染	有时表现为全血细胞减少和骨髓增生减低,可见肉芽肿、纤维化、骨髓坏死和噬血征象。结核分枝杆菌一般没有特征性肉芽肿。抗酸杆菌属于不典型分枝杆菌感染,其常被泡沫样巨噬细胞吞噬。如果考虑结核,应进行骨髓抗酸染色和培养
神经性厌食或长期饥饿	可表现为全血细胞减少、骨髓增生减低、脂肪细胞和造血细胞丢失,骨髓涂片背景物质增多,HE染色为浅粉色,吉姆萨染色亦可观察到
原发免疫性血小板减少(ITP)	部分AA患者初期仅表现为血小板减少,后期出现全血细胞减少,需与ITP相鉴别。这类AA患者骨髓增生减低、巨核细胞减少或消失。这种表现在ITP中并不常见。可用于鉴别早期AA及ITP
MonoMac综合征	骨髓增生减低同时外周血单核细胞减低或极度减低可能提示该诊断

注 AA:再生障碍性贫血;PNH:阵发性睡眠性血红蛋白尿症;GPI:糖基磷脂酰基醇;MDS:骨髓增生异常综合征;AML:急性髓系白血病;MonoMac综合征:分枝杆菌易感的单核细胞缺乏综合征

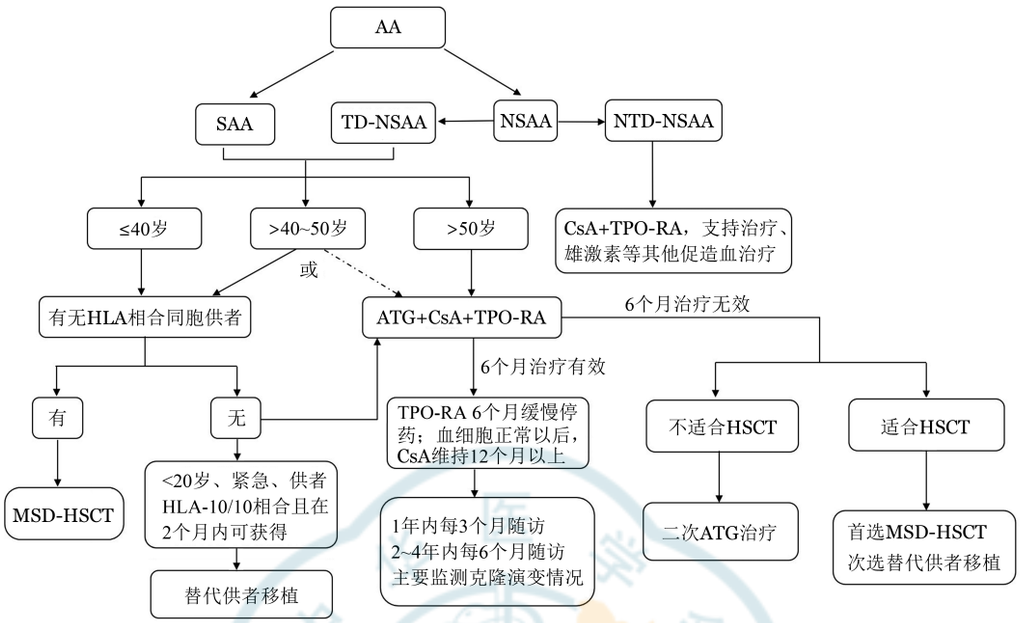
供者造血干细胞移植(MSD-HSCT)[12]。对无HLA相合同胞供者和年龄>40岁的患者首选免疫抑制治疗(IST)[抗胸腺/淋巴细胞球蛋白(ATG/ALG)+环孢素A(CsA)]联合促血小板生成素受体激动剂(TPO-RA)和(或)其他促造血的治疗方案[13-15];HLA相合无关供者造血干细胞移植(MUD-HSCT)或单倍体造血干细胞移植(Haplo-HSCT)目前提倡适用于IST无效的年轻SAA患者。对NTD-NSAA可采用CsA联合TPO-RA和(或)其他促造血治疗(图1)。

在一线治疗选择时,除了关注年龄、是否存在合并症、疾病的严重程度、造血干细胞移植合并症指数评分(HCT-CI)外,还需评价是否存在影响预后的其他因素。如存在多个IST预后良好因素(表3),则更倾向于一线使用IST联合TPO-RA治疗;如存在多项IST预后不良因素,如端粒显著缩短、不良基因突变(ASXL1、TP53、RUNX1、DNMT3A)、合并难以控制的活动性感染、从NSAA逐渐发展到SAA等,若条件允许尽量选择HSCT,TPO-RA联合IST选择需权衡利弊。无论如何,在TPO-RA联合IST疗效明显提升背景下,选择MUD-HSCT或Haplo-HSCT时,应充分衡量患者风险与获益[17]。

(二)支持疗法

1. 成分血输注:红细胞输注指征一般为HGB<60 g/L。老年(≥60岁)、代偿反应能力低(如伴有心、肺疾病)、需氧量增加(如感染、发热、疼痛等)、氧气供应缺乏加重(如失血、肺炎等)时红细胞输注指征可放宽为HGB≤80 g/L,尽量输注悬浮红细胞。

存在血小板消耗危险因素(感染、出血、使用抗生素或ATG/ALG等)者或SAA预防性血小板输注指征为PLT<20×10^9/L,病情稳定者为PLT<10×10^9/L。发生严重出血者则不受上述标准限制,应积极输注单采浓缩血小板悬液。重症感染(如败血症患者)



注　SAA：重型再生障碍性贫血；NSAA：非重型再生障碍性贫血；TD-NSAA：输血依赖非重型再生障碍性贫血；NTD-NSAA：非输血依赖非重型再生障碍性贫血；HLA：人类白细胞抗原；ATG：抗胸腺细胞球蛋白；CsA：环孢素A；TPO-RA：促血小板生成素受体激动剂；HSCT：造血干细胞移植；MSD-HSCT：同胞全相合HSCT

图1　再生障碍性贫血（AA）治疗路线图

表3　再生障碍性贫血（AA）免疫抑制治疗（IST）疗效良好的预测因素

预测因素
1. 年龄小
2. 病情较轻
3. 网织红细胞绝对值>25×10^9/L且淋巴细胞绝对值>1.0×10^9/L
4. 染色体异常+8或del（13q）
5. 存在PIGA基因突变或阵发性睡眠性血红蛋白尿克隆
6. 端粒长度不能预测血液学反应，但长端粒组IST后总生存率高
7. BCOR和BCORL1突变[16]

或ATG治疗期间，尽量维持PLT≥20×10^9/L。因产生抗血小板抗体而导致无效输注者应输注HLA配型相合的血小板。

粒细胞缺乏伴不能控制的细菌和真菌感染，广谱抗生素及抗真菌药物治疗无效可以考虑粒细胞输注治疗，建议连续输注3 d以上。治疗过程中预防及密切注意粒细胞输注相关不良反应，如输血相关性急性肺损伤、同种异体免疫反应及发热反应。

2. 其他保护措施：SAA患者应予保护性隔离，有条件者应入住层流病房；避免出血，防止外伤及剧烈活动；必要的心理护理。

3. 感染的预防和治疗：口腔护理及高压无菌饮食，必要时可预防性应用抗真菌药物。欲进行移植及ATG/ALG治疗者建议预防性应用抗细菌、抗病毒及抗真菌治疗。造血干细胞移植（HSCT）后需预防卡氏肺孢子菌感染，如用复方磺胺甲噁唑（SMZco）。由于已有一些报道提示接种疫苗可诱发BMF或AA复发，除非绝对必要否则不主张接种疫苗。AA患者发热同样遵循“中性粒细胞减少伴发热”的治疗原则处理。

4. 祛铁治疗：长期反复输血超过20 U和（或）血清铁蛋白水平高于1 000 μg/L的患者，有条件可进行肝脏、心脏MRI检查，明确铁过载程度。根据血细胞数量和脏器功能情况酌情祛铁治疗，以铁螯合剂为主，推荐应用去铁胺、地拉罗司。近期研究显示艾曲泊帕具有一定的祛铁作用，疗效与铁螯合剂相当，因此在IST联合TPO-RA治疗时代，祛铁治疗的策略是否面临改变尚需研究证实[18]。

（三）AA本病治疗

1. IST（ATG/ALG+CsA）联合促造血治疗：经NIH和RACE两个临床研究证实，已将IST联合TPO-RA方案确立为不适合移植SAA患者的一线治疗方案。

（1）ATG/ALG：目前ATG/ALG使用无年龄上限，对于＞60岁SAA患者需评估合并症以及患者一般情况是否适合应用。兔源ATG（法国）剂量为

2.5～3.5 mg·kg^{-1}·d^{-1}，猪源ALG（中国）剂量为20～30 mg·kg^{-1}·d^{-1}，连续使用5 d。输注之前均应按照相应药品制剂说明进行皮试和（或）静脉试验，试验阴性方可接受ATG/ALG治疗。每日用ATG/ALG时同步应用肾上腺糖皮质激素防止过敏反应。急性期不良反应包括：超敏反应、发热、僵直、皮疹、高血压或低血压及液体潴留。血清病反应（关节痛、肌痛、皮疹、轻度蛋白尿和血小板减少）一般出现在ATG/ALG治疗后1周左右，因此糖皮质激素应足量用至15 d，随后减量，一般2周后减完（总疗程4周），出现血清病反应者则静脉应用肾上腺糖皮质激素冲击治疗。

第1次ATG/ALG治疗无效或复发患者可选择HSCT或第2次ATG/ALG治疗。选择第2次ATG/ALG治疗，应与前次治疗间隔3～6个月，第2个疗程的ATG/ALG，宜尽可能采用动物种属来源与前次不同的ATG/ALG剂型，以减少过敏反应和严重血清病发生的风险。

（2）CsA：CsA联合ATG/ALG用于SAA时，CsA口服剂量为3～5 mg·kg^{-1}·d^{-1}，建议与ATG/ALG同时应用。CsA治疗AA的确切有效血药浓度并不明确，有效血药浓度窗较大，一般目标血药浓度（C0谷浓度）为成人150～250 μg/L，儿童酌减。临床可根据血药浓度及疗效调整CsA的应用剂量。CsA减量过早会增加复发风险，IST联合TPO-RA治疗SAA的方案中，CsA足量应用6个月或疗效达平台期后建议持续用药12～24个月后停药[13]。CsA主要的不良反应为消化道反应、齿龈增生、色素沉着、肌肉震颤、肝肾功能损害，少数出现头痛和血压变化；因此，服用CsA期间应定期检测血压、肝肾功能，出现上述不良反应可通过CsA减量或停药予以纠正。

（3）TPO-RA：TPO-RA包括海曲泊帕、艾曲泊帕、阿伐曲泊帕、罗米司亭等，其中海曲泊帕在我国获批难治成人SAA适应证，艾曲泊帕在美国获批治疗初诊及难治SAA，其他TPO-RA的临床研究均正在进行，目前多为探索性治疗。

艾曲泊帕在ATG应用第1天同时给药可获得最佳疗效，起始剂量为75 mg/d，根据疗效情况可每两周增加25 mg/d进行剂量爬坡，最大剂量为150 mg/d。血小板正常后缓慢减药，不要骤停，尤其对于老年及未达CR的患者[13,19]。海曲泊帕治疗难治成人SAA，推荐起始剂量7.5 mg/d，每2周加量2.5 mg/d，最大剂量15 mg/d[20]。艾曲泊帕及海曲泊帕均应空腹服用，避免与抗酸药物或含多价金属阳离子的食物，如奶制品等同服，或当有合并用药时根据药品说明书调整药物剂量。

艾曲泊帕及海曲泊帕最常见的不良反应为肝脏毒性，在治疗过程中应严密监测肝功能变化，目前没有证据表明艾曲泊帕增加克隆造血（CH）的发生率，但CH出现更早，需定期监测[14]。

对艾曲泊帕或海曲泊帕联合IST治疗SAA无效患者，可尝试TPO-RA之间的转换，如罗米司亭用于治疗艾曲泊帕无效的AA患者，起始剂量每周20 μg/kg，70%患者在3个月出现不同程度血液学反应[21]。但仍需更多临床研究数据指导用药选择。

2. 其他方案：

（1）其他免疫抑制剂：也有研究显示抗CD52单抗、他克莫司、雷帕霉素、环磷酰胺（Cy）等对于难治、复发SAA有效。

（2）其他促造血治疗：雄激素可以刺激骨髓红系造血，减轻女性AA患者月经期出血过多，且具有端粒调节作用，常用的雄激素包括：司坦唑醇、十一酸睾酮、达那唑等。国内研究显示重组人血小板生成素（TPO）及白细胞介素11（IL-11）联合IST也可治疗SAA[22]。G-CSF在IST治疗时代具有加速中性粒细胞恢复、协助控制感染等作用，到目前为止无证据表明G-CSF可增加克隆演变的风险。也有研究显示加用促红细胞生成素（EPO）可加速造血恢复[23]。

3. 随访：接受IST联合TPO-RA方案治疗的SAA患者应定期随访，以便及时评价疗效和不良反应。主要监测指标包括：①造血功能：血常规（带有网织红细胞绝对值）、骨髓检测（骨髓增生程度、形态学、流式细胞术检测细胞分群变化）；②免疫指标：T、B、NK及DC细胞各亚群数量及功能；③克隆演变：染色体、FISH、PNH克隆，MDS/急性髓系白血病（AML）二代测序、端粒长度；④用药不良反应：肝肾功能、电解质、血糖等。建议随访观察点为ATG/ALG用药后3个月、6个月、9个月、1年、1.5年、2年、2.5年、3年、3.5年、4年、5年、10年。

4. IST疗效的影响因素：在IST时代，IST治疗有效的预测因素见表3。TPO-RA的加入，提升了网织红细胞绝对值在（10～30）×10^9/L的SAA患者的疗效，使这部分患者疗效与网织红细胞绝对值＞30×10^9/L的SAA患者疗效相当。目前研究显示IST联合TPO-RA疗效的影响因素仍然限于年龄及残存骨髓造血指标[24-25]。

（四）HSCT

MSD-HSCT目前仍被认为是SAA与TD-NSAA

适合移植患者的首选治疗方案。对IST无效、适合移植但无HLA相合同胞供者的SAA与TD-NSAA患者，也可采用替代供者移植，包括：MUD-HSCT、Haplo-HSCT和脐血移植（CB-HSCT）。

1. MSD-HSCT：

（1）适用条件：年龄≤40岁、有HLA相合同胞供者的SAA与TD-NSAA患者；年龄超过40岁的SAA患者，在IST治疗失败后，条件允许也可采用MSD-HSCT。有经验的移植中心将MSD-HSCT一线治疗年龄放宽至≤50岁，IST治疗失败挽救治疗年龄放宽至50～60岁[26-27]。

（2）移植预处理和移植物抗宿主病（GVHD）预防：以大剂量Cy（200 mg·kg^{-1}·d^{-1}）和兔源ATG（10 mg·kg^{-1}·d^{-1}）作为预处理方案，使用甲氨蝶呤联合CsA预防GVHD。对于植入失败率相对较高的患者类型如≥40岁、病程长、重度输血患者，则给予强化预处理方案，在Cy-ATG基础上增加氟达拉滨（Flu）或白消安（BU）或小剂量全身放疗（TBI）以保证植入[28]。

（3）疗效及预后：研究显示以Flu/Cy/ATG（Flu 120 mg·m^{-2}·d^{-1}+Cy 120～200 mg·kg^{-1}·d^{-1}+ATG 10 mg·kg^{-1}·d^{-1}）或Cy/ATG（Cy 200 mg·kg^{-1}·d^{-1}+ATG 10 mg·kg^{-1}·d^{-1}）方案作为MSD预处理方案，5年生存率最高，可达91%。多因素相关分析显示影响MSD生存的预后因素为：受体年龄、是否使用含ATG的预处理方案[29]。

2. MUD-HSCT：多用于IST失败后的二线治疗，在一些特殊条件下，如预计IST疗效不佳或＜20岁的SAA或VSAA患者，骨髓库能找到HLA-10/10全合无关供者，情况紧急且在2个月之内能实施HSCT的，可将MUD-HSCT作为一线治疗[30]。MUD-HSCT治疗SAA常以Flu/Cy/ATG（Flu 120 mg·m^{-2}·d^{-1}+Cy 120～200 mg·kg^{-1}·d^{-1}+ATG 10 mg·kg^{-1}·d^{-1}）±低剂量TBI为预处理方案和甲氨蝶呤联合CsA预防GVHD。

3. Haplo-HSCT：Haplo-HSCT可作为缺乏MSD或MUD的补充治疗选择[31-33]，在有经验的移植中心Haplo-HSCT可用于缺乏MSD患者的一线治疗。

Haplo-HSCT多以BU/Cy/ATG（Bu 6.4 mg·kg^{-1}·d^{-1}+Cy 200 mg·kg^{-1}·d^{-1}+ATG 10 mg·kg^{-1}·d^{-1}）为预处理方案，以甲氨蝶呤联合CsA和吗替麦考酚酯预防GVHD；移植后环磷酰胺（PTCY）体系也在SAA的Haplo-HSCT取得进展[34]。

4. CB-HSCT：使用脐血作为SAA患者无关供者移植物来源的潜在优势是脐血获取容易，可及时进行移植，对HLA不相合具有更好的耐受性，当患者缺乏HLA相合无关供者时可以考虑行脐血移植。对于IST联合TPO-RA治疗失败患者，又缺乏HLA相合供者，只要脐血细胞计数足够（理想情况下总有核细胞＞4×10^7/kg），可作为一个有效的挽救性治疗手段[35]。

5. 其他探索性治疗：间充质干细胞治疗：间充质干细胞是骨髓造血微环境的重要成分，具有促进造血干细胞的分化和增殖、抗炎、修复损伤组织、调节机体免疫等作用，可促进移植造血干细胞的归巢，改善造血微环境，产生多种细胞因子，加速造血功能的恢复，降低GVHD的发生。

（五）特殊情况AA的处理

1. 伴有PNH克隆的AA患者的处理：在AA患者中可检测到少量PNH克隆，患者骨髓细胞减少但并不出现溶血，通常仅单核细胞和中性粒细胞单独受累，并且仅占很小部分。一些证据表明PNH克隆预示着IST反应更好，推荐对这些患者的处理同无PNH克隆的AA患者。但伴有明显PNH克隆（＞50%）及伴溶血临床及生化指标的AA患者慎用ATG/ALG治疗，以针对PNH治疗为主。

2. 妊娠AA患者的处理：AA可发生于妊娠过程中，有些患者需要支持治疗。AA患者妊娠后，疾病可能反复或进展，尤其对于未达到完全缓解的患者。对于妊娠AA患者主要是给予支持治疗，输注血小板维持患者PLT≥20×10^9/L。不推荐妊娠期使用ATG/ALG、HSCT或雄激素，可予CsA治疗。妊娠期间应该严密监测患者孕情、血常规和重要脏器功能。

3. 肝炎相关性AA的处理：肝炎相关性AA大都发生在肝炎发生后的2～3个月内。如果AA发病前有黄疸史（通常为发病前的2～3个月）则提示可能为肝炎相关性AA。肝功能检查有利于发现肝炎相关性AA。肝炎相关性AA的肝炎病原学检查可为阴性。针对肝炎应该检测甲肝抗体、乙肝表面抗原、丙肝抗体及EBV。合并肝炎的AA病情一般较重，对治疗反应差，预后不良。在目前国内可获得的TPO-RA中，阿伐曲泊帕虽然现阶段临床适应证为“择期行诊断性操作或者手术的慢性肝病相关血小板减少症的成年患者”，但对于肝炎相关AA或AA伴有肝功能异常者，可尝试使用阿伐曲泊帕治疗。

4. 老年AA的治疗：IST联合TPO-RA为首选治

疗，部分有MSD的患者，条件允许可以考虑HSCT。尽管对于SAA或TD-NSAA患者，ATG联合CsA比单用CsA疗效更好；但是，对于老年患者，ATG治疗相关毒副作用更大、风险更高，因此是否应用仍需谨慎，如应用可酌情减量。研究显示CsA联合TPO-RA一线治疗可使无法接受ATG治疗的AA患者获益。其他治疗包括单药CsA、雄激素及阿仑单抗。不耐受或拒绝IST的患者也可考虑中医中药及支持对症治疗。

四、AA的疗效标准

1. SAA的IST疗效标准：

完全缓解（CR）：HGB > 100 g/L；ANC > 1.5×10^9/L；PLT > 100×10^9/L。

部分缓解（PR）：脱离成分血输注，不再符合SAA诊断标准。

无效（NR）：仍满足SAA诊断标准。

2. NSAA的IST疗效标准：

完全缓解（CR）：同SAA疗效标准。

部分缓解（PR）：脱离成分血输注（若既往输血依赖），或至少一系细胞数目增加两倍或达正常，或任何一系血细胞基线水平上升：HGB > 30 g/L（如治疗前 < 60 g/L）、ANC > 0.5×10^9/L（如治疗前 < 0.5×10^9/L）、PLT > 20×10^9/L（如治疗前 < 20×10^9/L）。

无效（NR）：疾病进展，或未能达到上述有效指标。

（执笔：付蓉、李莉娟）

参与指南讨论的专家（按照姓氏笔画排序）：马艳萍（山西医科大学第二医院）；王化泉（天津医科大学总医院）；王顺清（广州市第一人民医院）；王荷花（中山大学附属一院）；王婷（天津医科大学总医院）；文钦（陆军军医大学附属新桥医院）；田发青（深圳龙岗区人民医院）；付蓉（天津医科大学总医院）；任金海（河北医科大学第二医院）；刘红（南通大学附属医院）；刘辉（北京医院）；刘立民（苏州大学附属第一医院）；刘春燕（天津医科大学总医院）；刘容容（广西医科大学第一附属医院）；李静（西安交通大学第一附属医院）；李燕（新疆维吾尔自治区人民医院）；李文倩（青海省人民医院）；李莉娟（兰州大学第二医院）；李德鹏（徐州医科大学附属医院）；杨岩（吉林大学第一医院）；何川（四川大学华西医院）；佟红艳（浙江大学附属第一医院）；张凤奎（中国医学科学院血液病医院）；张连生（兰州大学第二医院）；陈彤（上海复旦大学附属华山医院）；苗瞄（苏州大学附属第一医院）；林圣云（浙江省中医院）；林丽娥（海南省人民医院）；郑波（宁夏医科大学总医院）；郑以州（中国医学科学院血液病医院）；孟凡凯（华中科技大学同济医学院附属同济医院）；赵明峰（天津市第一中心医院）；施均（中国医学科学院血液病医院）；姜中兴（郑州大学附属第一医院）；洪梅（华中科技大学同济医学院附属协和医院）；祝亮方（福建医科大学协和医院）；贾晋松（北京大学人民医院）；徐雅靖（中南大学湘雅医院）；高晓宁（解放军总医院）；黄健（浙江大学附属第一医院）；常春康（上海市第六人民医院）；董宝侠（空军军医大学西京医院）；韩冰（北京协和医院）；曾云（昆明医科大学第一附属医院）；戴敏（南方医科大学南方医院）

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·标准与讨论·

再生障碍性贫血诊断与治疗中国专家共识(2017年版)

中华医学会血液学分会红细胞疾病(贫血)学组

Chinese expert consensus on the diagnosis and treatment of aplastic anemia (2017) *Red Blood Cell Disease (Anemia) Group, Chinese Society of Hematology, Chinese Medical Association*

Corresponding author: Shao Zonghong, Department of Hematology, General Hospital, Tianjin Medical University, Tianjin 300052, China. Email: shaozonghong@sina.com; Zhang Liansheng, Department of Hematology, Lanzhou University Second Hospital, Lanzhou 730030, China. Email: zhangliansheng@medmail.com.cn

为进一步提高我国再生障碍性贫血(AA)的诊治水平,中华医学会血液学分会红细胞疾病(贫血)学组在《再生障碍性贫血诊断治疗专家共识》(2010版)的基础上,参考国外诊治指南及近年相关文献,广泛征求专家建议和意见,重新制订了以下AA诊断与治疗新版中国专家共识。

一、AA定义及发病机制

AA是一种骨髓造血衰竭(BMF)综合征。其年发病率在我国为0.74/10万人口,可发生于各年龄组,老年人发病率较高,男、女发病率无明显差异。AA分为先天性及获得性。目前认为T淋巴细胞异常活化、功能亢进造成骨髓损伤在原发性获得性AA发病机制中占主要地位,新近研究显示遗传背景在AA发病及进展中也可能发挥一定作用,如端粒酶基因突变,也有部分病例发现体细胞突变。先天性AA罕见,主要为范可尼贫血(FA)、先天性角化不良(DKC)、先天性纯红细胞再生障碍(DBA)、Shwachmann-Diamond综合征(SDS)等。绝大多数AA属获得性,故本指南主要讨论原发性获得性AA。

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通信作者:邵宗鸿,天津医科大学总医院血液科,300052,Email:shaozonghong@sina.com;张连生,兰州大学第二医院,730030,Email:zhangliansheng@medmail.com.cn

二、AA的诊断建议

(一)诊断AA的实验室检测项目

1. 必需检测项目:(1)血常规检查:白细胞计数及分类、红细胞计数及形态、血红蛋白(HGB)水平、网织红细胞百分比和绝对值、血小板计数(PLT)和形态。(2)多部位骨髓穿刺:至少包括髂骨和胸骨。骨髓涂片分析:造血细胞增生程度;粒、红、淋巴系细胞形态和阶段百分比;巨核细胞数目和形态;小粒造血细胞面积;是否有异常细胞等。(3)骨髓活检:至少取2 cm骨髓组织(髂骨)标本用以评估骨髓增生程度、各系细胞比例、造血组织分布(有无灶性$CD34^+$细胞分布等)情况,以及是否存在骨髓浸润、骨髓纤维化等。(4)流式细胞术检测骨髓$CD34^+$细胞数量。(5)肝、肾、甲状腺功能,其他生化,病毒学(包括肝炎病毒、EBV、CMV等)及免疫固定电泳检查。(6)血清铁蛋白、叶酸和维生素B_{12}水平。(7)流式细胞术检测阵发性睡眠性血红蛋白尿症(PNH)克隆(CD55、CD59、Flaer)。(8)免疫相关指标检测:T细胞亚群(如$CD4^+$、$CD8^+$、Th1、Th2、Treg等)及细胞因子(如IFN-γ、IL-4、IL-10等)、自身抗体和风湿抗体、造血干细胞及大颗粒淋巴细胞白血病相关标志检测。(9)细胞遗传学:常规核型分析、荧光原位杂交[del(5q33)、del(20q)等]以及遗传性疾病筛查(儿童或有家族史者推荐做染色体断裂试验),胎儿血红蛋白检测。(10)其他:心电图、肺功能、腹部超声、超声心动图及其他影像学检查(如胸部X线或CT等),以评价其他原因导致的造血异常。

2. 可选检测项目:有条件的医院可开展以下项目:(1)骨髓造血细胞膜自身抗体检测;(2)端粒长度及端粒酶活性检测、端粒酶基因突变检测、体细胞基因突变检测。

(二)AA诊断标准

1. 血常规检查:全血细胞(包括网织红细胞)减少,淋巴细胞比例增高。至少符合以下三项中两项:HGB<100 g/L;PLT<50×10^9/L;中性粒细胞绝对值(ANC)<1.5×10^9/L。

2. 骨髓穿刺：多部位（不同平面）骨髓增生减低或重度减低；小粒空虚，非造血细胞（淋巴细胞、网状细胞、浆细胞、肥大细胞等）比例增高；巨核细胞明显减少或缺如；红系、粒系细胞均明显减少。

3. 骨髓活检（髂骨）：全切片增生减低，造血组织减少，脂肪组织和（或）非造血细胞增多，网硬蛋白不增加，无异常细胞。

4. 除外检查：必须除外先天性和其他获得性、继发性BMF，见表1。

（三）AA严重程度确定（Camitta标准）

1. 重型AA诊断标准：（1）骨髓细胞增生程度<正常的25%；如≥正常的25%但<50%，则残存的造血细胞应<30%。（2）血常规：需具备下列三项中的两项：ANC<0.5×10^9/L；网织红细胞绝对值<20×10^9/L；PLT<20×10^9/L。（3）若ANC<0.2×10^9/L为极重型AA。

2. 非重型AA诊断标准：未达到重型标准的AA。

（四）AA鉴别诊断

AA应与其他引起全血细胞减少的疾病相鉴别，见表1。AA属于BMF。BMF可以分为先天性和获得性两种，而获得性BMF又分为原发性和继发性。

1. 原发性BMF：原发性BMF主要包括：（1）源于造血干细胞质量异常的BMF，如PNH和骨髓增生异常综合征（MDS）；（2）自身免疫介导的BMF，其中又包括细胞免疫介导的BMF（如AA）和自身抗体介导的BMF；（3）意义未明的血细胞减少（ICUS）[包括非克隆性ICUS、意义未明克隆性血细胞减少（CCUS）]这些情况可以是某特定疾病的过渡阶段，可发展为MDS或其他血液病，也可能是尚未认知的某疾病。

2. 继发性BMF：造成继发性BMF的因素较多，主要包括：（1）造血系统肿瘤，如毛细胞白血病（HCL）、T细胞型大颗粒淋巴细胞白血病（T-LGLL）、多发性骨髓瘤（MM）等；（2）其他系统肿瘤浸润骨髓；（3）骨髓纤维化；（4）严重营养性贫血；（5）急性造血功能停滞；（6）肿瘤性疾病因放化疗所致骨髓抑制等。

三、AA的治疗建议

（一）支持疗法

表1　全血细胞减少和骨髓低增生的其他疾病

疾病或临床表现	鉴别要点
PNH相关（AA/PNH）	依据疾病及PNH向AA转化的阶段不同，患者的临床表现不同。检测外周血红细胞和白细胞表面GPI锚链蛋白可以鉴别
低增生性MDS/AML	低增生性MDS具备如下特点：粒系、巨核系增生减低，外周血、骨髓涂片和骨髓活检中存在幼稚细胞。骨髓活检标本中，网状纤维、$CD34^+$细胞增加以及较多的残存造血面积提示为低增生性MDS而非AA。若存在前体细胞异常定位（ALIP）则更加提示MDS。红系病态造血在AA中非常常见，不能据此鉴别MDS和AA
自身抗体介导的全血细胞减少	包括Evans综合征等。可检测到外周成熟血细胞的自身抗体或骨髓未成熟血细胞的自身抗体，患者可有全血细胞减少并骨髓增生减低，但外周血网织红细胞或中性粒细胞比例往往不低甚或偏高，骨髓红系细胞比例不低且易见“红系造血岛”，Th1/Th2降低（Th2细胞比例增高）、$CD5^+$ B细胞比例增高，血清IL-4和IL-10水平增高，对糖皮质激素和（或）大剂量静脉滴注丙种球蛋白的治疗反应较好
霍奇金淋巴瘤或非霍奇金淋巴瘤	可表现为全血细胞减少、骨髓增生减低、骨髓涂片可见局部淋巴瘤细胞浸润。AA患者淋巴细胞显著增高，但系正常淋巴细胞，可通过免疫分型和基因重排检测与淋巴瘤细胞进行区分。其他如脾肿大等特征也可作为鉴别AA与淋巴瘤的依据
原发性骨髓纤维化	原发性骨髓纤维化常伴随出现泪滴样异常红细胞、幼稚红细胞、脾肿大。骨髓纤维化不合并脾肿大的患者则提示有可能是继发于其他恶性肿瘤
分枝杆菌感染	有时表现为全血细胞减少和骨髓增生减低，可见肉芽肿、纤维化、骨髓坏死和嗜血征象。结核分枝杆菌一般没有特征性肉芽肿。抗酸杆菌属于不典型分枝杆菌感染，其常被泡沫样巨噬细胞吞噬。如果考虑结核，应进行骨髓抗酸染色和培养
神经性厌食或长期饥饿	可表现为全血细胞减少、骨髓增生减低、脂肪细胞和造血细胞丢失，骨髓涂片背景物质增多，HE染色为浅粉色，吉姆萨染色亦可观察到
原发免疫性血小板减少症（ITP）	部分AA患者初期仅表现为血小板减少，后期出现全血细胞减少，需与ITP相鉴别。这类AA患者骨髓增生减低、巨核细胞减少或消失。这种表现在ITP中并不常见。可用于鉴别早期AA及ITP
MonoMac综合征	骨髓增生减低同时外周血单核细胞减低或极度减低可能提示该诊断

注：AA：再生障碍性贫血；PNH：阵发性睡眠性血红蛋白尿症；GPI：糖基磷脂酰基醇；MDS：骨髓增生异常综合征；AML：急性髓系白血病；MonoMac综合征：分枝杆菌易感的单核细胞缺乏综合征

1. 成分血输注：红细胞输注指征一般为HGB<60 g/L。老年（≥60岁）、代偿反应能力低（如伴有心、肺疾患）、需氧量增加（如感染、发热、疼痛等）、氧气供应缺乏加重（如失血、肺炎等）时红细胞输注指征可放宽为HGB≤80 g/L），尽量输注红细胞悬液。拟行异基因造血干细胞移植者应输注辐照或过滤后的红细胞和血小板悬液。存在血小板消耗危险因素者[感染、出血、使用抗生素或抗胸腺/淋巴细胞球蛋白（ATG/ALG）等]或重型AA预防性血小板输注指征为PLT<20×10^9/L，病情稳定者为PLT<10×10^9/L。发生严重出血者则不受上述标准限制，应积极输注单采浓缩血小板悬液。因产生抗血小板抗体而导致无效输注者应输注HLA配型相合的血小板。粒细胞缺乏伴不能控制的细菌和真菌感染，广谱抗生素及抗真菌药物治疗无效可以考虑粒细胞输注治疗。粒细胞寿命仅6~8 h，建议连续输注3 d以上。治疗过程中预防及密切注意粒细胞输注相关不良反应，如输血相关性急性肺损伤、同种异体免疫反应及发热反应。

2. 其他保护措施：重型AA患者应予保护性隔离，有条件者应入住层流病房；避免出血，防止外伤及剧烈活动；必要的心理护理。需注意饮食卫生，可预防性应用抗真菌药物。欲进行移植及ATG/ALG治疗者建议给予预防性应用抗细菌、抗病毒及抗真菌治疗。造血干细胞移植后需预防卡氏肺孢子菌感染，如用复方磺胺甲恶唑（SMZco），但ATG/ALG治疗者不必常规应用。

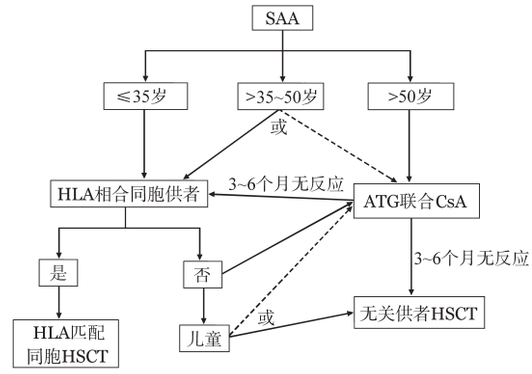
3. 感染的治疗：AA患者发热应按“中性粒细胞减少伴发热”的治疗原则来处理。

4. 祛铁治疗：长期反复输血超过20 U和（或）血清铁蛋白水平增高达铁过载标准的患者，可酌情予祛铁治疗。

5. 疫苗接种：已有一些报道提示接种疫苗可导致BMF或AA复发，除非绝对需要否则不主张接种疫苗。

（二）AA本病治疗

AA一旦确诊，应明确疾病严重程度，尽早治疗。重型AA的标准疗法是对年龄>35岁或年龄虽≤35岁但无HLA相合同胞供者的患者首选ATG/ALG和环孢素A（cyclosporin A，CsA）的免疫抑制治疗（IST）；对年龄≤35岁且有HLA相合同胞供者的重型AA患者，如无活动性感染和出血，首选HLA相合同胞供者造血干细胞移植。HLA相合无关供者造血干细胞移植仅用于ATG/ALG和CsA治疗无效的年轻重型AA患者。造血干细胞移植前必须控制出血和感染。输血依赖的非重型AA可采用CsA联合促造血（雄激素、造血生长因子）治疗，如治疗6个月无效则按重型AA治疗。非输血依赖的非重型AA，可应用CsA和（或）促造血治疗（图1）。



注：CsA：环孢素A；HSCT：造血干细胞移植

图1 重型再生障碍性贫血（SAA）治疗选择

1. IST：

（1）ATG/ALG联合CsA的IST适用范围：无HLA相合同胞供者的重型或极重型AA患者；输血依赖的非重型AA患者；CsA治疗6个月无效患者。

（2）ATG/ALG：兔源ATG/ALG（法国、德国产）剂量为3~4 mg·kg^{-1}·d^{-1}，猪源ALG（中国产）剂量为20~30 mg·kg^{-1}·d^{-1}。ATG/ALG需连用5 d，每日静脉输注12~18 h。输注之前均应按照相应药品制剂说明进行皮试和（或）静脉试验，试验阴性方可接受ATG/ALG治疗。每日用ATG/ALG时同步应用肾上腺糖皮质激素防止过敏反应。急性期不良反应包括超敏反应、发热、僵直、皮疹、高血压或低血压及液体潴留。患者床旁应备气管切开包、肾上腺素。用药期间维持PLT>10×10^9/L，因ATG/ALG具有抗血小板活性的作用，血小板悬液输注需要量可能会增加。血清病反应（关节痛、肌痛、皮疹、轻度蛋白尿和血小板减少）一般出现在ATG/ALG治疗后1周左右，因此糖皮质激素应足量用至15 d，随后减量，一般2周后减完（总疗程4周），出现血清病反应者则静脉应用肾上腺糖皮质激素冲击治疗。第1次ATG/ALG治疗无效或复发患者2次治疗可选择HLA相合无关供者造血干细胞移植或第2次ATG/ALG治疗。选择第2次IST，与前次治疗应间隔3~6个月，第2个疗程的ATG/ALG，宜尽可能采用动物种属来源于前次不同的ATG/ALG剂型，以减少发

生过敏反应和严重血清病风险。

（3）CsA：CsA联合ATG/ALG用于重型AA时，CsA口服剂量为3~5 mg·kg^{-1}·d^{-1}，可以与ATG/ALG同时应用，或在停用糖皮质激素后，即ATG/ALG开始后4周始用。CsA可用于非重型AA的治疗。CsA治疗AA的确切有效血药浓度并不明确，有效血药浓度窗较大，一般目标血药浓度（谷浓度）为成人100~200 μg/L、儿童100~150 μg/L。临床可根据药物浓度及疗效调整CsA的应用剂量。CsA的主要不良反应是消化道反应、齿龈增生、色素沉着、肌肉震颤、肝肾功能损害，极少数出现头痛和血压变化，多数患者症状轻微或经对症处理减轻，必要时减量甚至停药。CsA减量过快会增加复发风险，一般建议逐渐缓慢减量，疗效达平台期后持续服药至少12个月。服用CsA期间应定期监测血压、肝肾功能。

（4）IST在老年患者中的应用：ATG治疗AA无年龄限制，但老年AA患者治疗前要评估合并症。ATG/ALG治疗老年AA患者时，出血、感染和心血管事件发生风险高于年轻患者，因此需要注意老年患者的心功能、肝功能、血脂、糖耐量等方面问题。鉴于肾毒性和高血压的风险，建议老年AA患者的CsA治疗血药谷浓度在100~150 μg/L。

（5）促造血治疗：雄激素可以刺激骨髓红系造血，减轻女性患者月经期出血过多，是AA治疗的基础促造血用药。其与CsA配伍，治疗非重型AA有一定疗效。一般应用司坦唑醇、十一酸睾酮或达那唑，应定期复查肝功能。据报道GM-CSF、G-CSF配合免疫抑制剂使用可发挥促造血作用。也有人主张加用红细胞生成素（EPO）。艾曲波帕（Eltrombopag）是血小板受体激动剂，美国FDA已批准用于难治性重型AA的治疗。据报道重组人血小板生成素（TPO）及白细胞介素11（IL-11）也可与IST联合有效治疗AA。

（6）随访：接受ATG/ALG和CsA治疗的患者应密切随访，定期检查以便及时评价疗效和不良反应（包括演变为克隆性疾病如PNH、MDS和急性髓系白血病等）。建议随访观察点为ATG/ALG用药后3个月、6个月、9个月、1年、1.5年、2年、2.5年、3年、3.5年、4年、5年、10年。

（7）IST疗效影响因素：见表2。

2. HLA相合同胞供者造血干细胞移植：

（1）适用条件：年龄≤35岁、有HLA相合同胞供者的重型或极重型AA患者；年龄超过35岁的重型AA患者，在ATG/ALG联合CsA治疗失败后，也可采用HLA相合同胞供者造血干细胞移植。

表2　预测再生障碍性贫血（AA）免疫抑制治疗（IST）有效的因素（国外研究显示）

1. 年龄小疗效好
2. 病情较轻疗效好
3. 网织红细胞绝对值>25×10^9/L且淋巴细胞绝对值>1.0×10^9/L疗效好
4. 染色体异常+8或del（13q）疗效好
5. 存在阵发性睡眠性血红蛋白尿克隆疗效好
6. 端粒长度不能预测血液学反应，但长端粒组IST后总生存率高

（2）干细胞数量：回输单个核细胞建议至少3×10^8/kg体重，CD34$^+$细胞至少3×10^6/kg体重。采用含骨髓移植物。

（3）移植预处理和移植后IST：年龄<30岁患者，标准预处理方案是大剂量环磷酰胺50 mg·kg^{-1}·d^{-1}×4 d（−5~−2 d）和兔源ATG。移植后CsA等基础免疫抑制剂应用建议1年后缓慢减停。

3. HLA相合的无关供者造血干细胞移植：

（1）适用条件：需同时满足以下条件：①有HLA完全相合（在DNA水平Ⅰ类抗原和Ⅱ类抗原）供者；②年龄<50岁（50~60岁间，须一般状况良好）；③重型或极重型AA患者；④无HLA相合的同胞供者；⑤至少1次ATG/ALG和CsA治疗失败；⑥造血干细胞移植时无活动性感染和出血。

（2）预处理方案：年轻患者推荐使用环磷酰胺300 mg·m^{-2}·d^{-1}×4 d；氟达拉滨30 mg·m^{-2}·d^{-1}×4 d；兔源ATG；CsA 1 mg·kg^{-1}·d^{-1}（−6~−2 d），2 mg·kg^{-1}·d^{-1}（−1~+20 d），其后改为8 mg·kg^{-1}·d^{-1}口服；采用含骨髓移植物。移植后CsA等基础免疫抑制剂应用建议1年后缓慢减停。目前造血干细胞移植治疗重型AA建议在儿童及年轻患者中避免含照射的预处理方案，即使是低剂量照射也应避免，代之以氟达拉滨。老年患者给予低剂量照射可能对降低排斥反应有益。亦有研究显示单倍体造血干细胞移植应用于SAA治疗有效。

4. 其他免疫抑制剂：

（1）大剂量环磷酰胺：由于大剂量环磷酰胺（45 mg·kg^{-1}·d^{-1}×4 d）的高致死率和严重毒性，不推荐其用于不进行造血干细胞移植的初诊患者或ATG/ALG联合CsA治疗失败的AA患者。

（2）霉酚酸酯（MMF）：对于该药的研究主要集中于治疗难治性AA，但多个中心研究表明MMF对难治性AA无效。

（3）普乐可复（FK506）：与CsA抑制T细胞活化

的信号通路相同但作用更强、肾毒性更小，且无齿龈增生，因此被用来替换CsA用于AA的治疗，初步效果令人鼓舞，值得临床探索。

（4）雷帕霉素：在抑制T细胞免疫方面与CsA有协同作用，但最新研究显示，在ATG/ALG联合CsA基础上加用雷帕霉素不能提高患者的治疗反应率。雷帕霉素联合CsA治疗难治、复发AA的临床研究正在进行。

（5）抗CD52单抗：已有部分学者应用CD52单抗治疗复发SAA，但仍缺乏大样本的临床研究来肯定该药物疗效，故目前仅推荐考虑作为二线方案，应用于治疗复发SAA。

（三）出现异常克隆AA患者的处理

少部分AA患者在诊断时存在细胞遗传学克隆异常，常见有+8、+6、13号染色体异常。一般异常克隆仅占总分裂象的很小部分，可能为一过性，可以自行消失。一些研究显示有无上述遗传学异常的AA患者对IST的反应类似。有异常核型的AA患者应该每隔3~6个月行1次骨髓细胞遗传学分析，异常分裂象增多提示疾病转化。

（四）伴有明显PNH克隆的AA患者的处理

在AA患者中可检测到少量PNH克隆，患者骨髓细胞减少但并不出现溶血。通常仅单核细胞和中性粒细胞单独受累，并且仅占很小部分。推荐对这些患者的处理同无PNH克隆的AA患者。伴有明显PNH克隆（>50%）及伴溶血临床及生化指标的AA患者慎用ATG/ALG治疗。AA-PNH或PNH-AA综合征患者的治疗以PNH为主，兼顾AA。推荐对于PNH克隆进行长期监测。

（五）妊娠AA患者的处理

AA可发生于妊娠过程中，有些患者需要支持治疗。AA患者妊娠后，疾病可能进展。对于妊娠AA患者主要是给予支持治疗，输注血小板维持患者PLT≥20×10^9/L。不推荐妊娠期使用ATG/ALG，可予CsA治疗。妊娠期间应该严密监测患者孕情、血常规和重要脏器功能。

（六）肝炎相关性AA的处理

肝炎相关性AA大都在肝炎发生后的2~3个月内发病。如果发病前有黄疸史（通常为发病前的2~3个月）则提示可能为肝炎相关性AA。肝功能检查有利于发现肝炎相关性AA。肝炎相关性AA的肝炎病原学检查可为阴性。应该检测甲肝抗体、乙肝表面抗原、丙肝抗体及EBV。合并肝炎的AA病情一般较重，对治疗反应差，预后不良。

（七）老年AA的治疗

IST仍为首选，部分有同基因供者的患者可以考虑造血干细胞移植。尽管对于非重型AA患者，ATG联合CsA比单用CsA疗效更好，但是，对于老年患者ATG治疗的相关毒副作用更大、风险更高，因此是否应用仍需谨慎。其他治疗包括单药CsA、雄激素及阿仑单抗。不耐受或拒绝IST的患者可给予中医中药等支持对症治疗。

四、AA的疗效标准

1. 基本治愈：贫血和出血症状消失，HGB男性达120 g/L、女性达110 g/L，ANC>1.5×10^9/L，PLT>100×10^9/L，随访1年以上未复发。

2. 缓解：贫血和出血症状消失，HGB男性达120 g/L、女性达100 g/L，WBC达3.5×10^9/L左右，PLT也有一定程度增加，随访3个月病情稳定或继续进步。

3. 明显进步：贫血和出血症状明显好转，不输血，HGB较治疗前1个月内常见值增长30 g/L以上，并能维持3个月。

判定以上三项疗效标准者，均应3个月内不输血。

4. 无效：经充分治疗后，症状、血常规未达明显进步。

（执笔：付蓉）

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国家卫生健康委办公厅

1 概述

再生障碍性贫血(aplastic anemia, AA)是一组以骨髓有核细胞增生减低和外周两系或三系(全血)血细胞减少为特征的骨髓衰竭性疾病,属于骨髓造血衰竭(bone marrow failure, BMF)综合征的一种。其年发病率在我国为0.74/10万人口,可发生于各年龄组,男、女发病率无明显差异。

BFM 分为先天性和获得性两大类。先天性BFM 主要包括 Fanconi 贫血、先天性角化不良、Shwachman-Diamond 综合征、Diamond-Blackfan 贫血和先天性无巨核细胞性血小板减少症等。获得性BMF 又分为原发性和继发性两类。

原发性BMF主要包括:①源于造血干细胞质量异常的BMF,如阵发性睡眠性血红蛋白尿(paroxysmal nocturnal hemoglobinuria, PNH)和骨髓增生异常综合征(myelodysplastic syndrome, MDS);②自身免疫介导的BMF,其中又包括细胞免疫介导的BMF(如AA)和自身抗体介导的BMF;③意义未明的血细胞减少(包括非克隆性血细胞减少、意义未明克隆性血细胞减少),这些情况可以是某特定疾病的过渡阶段,可发展为MDS 或其他血液病,也可能是尚未认知的某疾病。

继发性主要包括:①造血系统肿瘤,如毛细胞白血病、T 细胞型大颗粒淋巴细胞白血病、多发性骨髓瘤等;②其他系统肿瘤浸润骨髓;③骨髓纤维化;④严重营养性贫血;⑤急性造血功能停滞;⑥肿瘤性疾病因放化疗所致骨髓抑制等;⑦药物、放射损伤、病毒感染等。

2 适用范围

本规范主要适用于经骨髓细胞形态学及骨髓病理、免疫表型、细胞遗传学、分子遗传学等检查确诊的儿童和青少年AA 患儿。

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3 诊断

3.1 临床表现 主要表现为贫血、出血、感染等血细胞减少的相应临床表现。一般无肝、脾、淋巴结肿大。

3.2 实验室检查

3.2.1 必需检测项目 ①血常规检查:白细胞计数及分类、红细胞计数及形态、血红蛋白水平、网织红细胞百分比和绝对值、血小板计数和形态。②多部位骨髓穿刺:首选髂骨骨髓穿刺,如髂骨不能诊断可进行胸骨骨髓穿刺,1 岁以下婴儿建议胫骨穿刺。骨髓涂片分析:造血细胞增生程度;粒、红、淋巴系细胞形态和阶段百分比;巨核细胞数目和形态;小粒造血细胞面积;是否有异常细胞等。③骨髓活检:至少取2 cm 骨髓组织(髂骨)标本用以评估骨髓增生程度、各系细胞比例、造血组织分布(有无灶性CD^{34+}细胞分布等)情况,以及是否存在骨髓浸润、骨髓纤维化等。④流式细胞术检测骨髓CD^{34+}细胞数量。⑤肝、肾、甲状腺功能,其他生化,病毒学(包括肝炎病毒、EB病毒、巨细胞病毒等)及免疫固定电泳检查。⑥血清铁蛋白、叶酸和维生素B_{12}水平。⑦流式细胞术检测PNH 克隆(CD^{55}、CD^{59}、Flaer)。⑧免疫相关指标检测:T 细胞亚群(如CD^{4+}、CD^{8+}、Th1、Th2、Treg 等)及细胞因子(如干扰素-γ、白介素-4、白介素-10等)、自身抗体和风湿抗体、造血干细胞及大颗粒淋巴细胞白血病相关标志检测。⑨细胞遗传学:常规核型分析、荧光原位杂交[del(5q33)、del(20q)、-7、+8 等]以及遗传性疾病筛查(儿童或有家族史者推荐做染色体断裂试验),胎儿血红蛋白检测。⑩基因检查:高度怀疑为先天性BMF或有阳性家族史者建议进行先天性骨髓衰竭性疾病相关的基因检查。⑪其他:心电图、腹部超声、超声心动图及其他影像学检查(如胸部X 线或CT 等),以评价其他原因导致的造血异常。

3.2.2　可选检测项目（有条件的医院可开展以下项目）①骨髓造血细胞膜自身抗体检测；②端粒长度及端粒酶活性检测、端粒酶基因突变检测；③体细胞基因突变检测；④血TPO 水平测定；⑤单核苷酸多态性芯片核型分析检测染色体不平衡缺陷。

3.3　诊断标准

3.3.1　血常规检查　红细胞、粒细胞和血小板减少，校正后的网织红细胞＜1%。至少符合以下3 项中的2项：①血红蛋白＜100 g/L；②血小板＜100×10^9/L；③中性粒细胞绝对值＜1.5×10^9/L（如为两系减少则必须包含血小板减少）。

3.3.2　骨髓穿刺检查　骨髓有核细胞增生程度活跃或减低，骨髓小粒造血细胞减少，非造血细胞（淋巴细胞、网状细胞、浆细胞、肥大细胞等）比例增高；巨核细胞明显减少或缺如，红系、粒系可明显减少。由于儿童不同部位造血程度存在较大差异，骨髓穿刺部位推荐首选髂骨或胫骨（年龄＜1岁者）。

3.3.3　骨髓活检　骨髓有核细胞增生减低，巨核细胞减少或缺如，造血组织减少，脂肪和或非造血细胞增多，无纤维组织增生，网状纤维染色阴性，无异常细胞浸润。如骨髓活检困难可行骨髓凝块病理检查。

3.3.4　除外检查　必须除外先天性和其他获得性、继发性BMF。

3.4　分型诊断标准　符合上述AA 诊断标准者，根据骨髓病理及外周血细胞计数分型。

3.4.1　重型再生障碍性贫血（severe aplastic anemia，SAA）　骨髓有核细胞增生程度25%～50%，残余造血细胞少于30%或有核细胞增生程度低于25%；外周血象至少符合以下3项中的2 项：①中性粒细胞绝对值＜0.5×10^9/L；②血小板计数＜20×10^9/L；③网织红细胞绝对值＜20×10^9/L，或校正后的网织红细胞＜1%。

3.4.2　极重型再生障碍性贫血（very severe aplastic anemia，VSAA）除满足SAA 条件外，中性粒细胞绝对值＜0.2×10^9/L。

3.4.3　非重型再生障碍性贫血（non-severe aplastic anemia，NSAA）　未达到SAA 和VSAA 诊断标准。需参考有关成份输血指征，确定是否属于“依赖输血NSAA”。

3.5　鉴别诊断

3.5.1　先天性BMF　诊断获得性再障必先除外先天性BMF。Fanconi 贫血、先天性角化不良和Shwachman-Diamond 综合征是最为常见的先天性BFM类型，其中Fanconi贫血 最为多见。由于典型的先天性角化不良 存在明显的色素沉着、趾/指甲萎缩和口腔黏膜白斑等三联征，不难诊断。但有些先天性角化不良的三联征最初并不典型，而是随年龄的增长而逐渐显现，因此该部分病例需高度重视家族史的询问。Shwachman-Diamond综合征多于婴儿期起病，常伴随严重中性粒细胞减少或伴有胰腺外分泌功能异常导致的消化不良症状，故不难与获得性AA 鉴别。而对于不存在明显躯体畸形或智力落后的Fanconi贫血，丝裂霉素C 诱导的染色体断裂试验和二代测序的先天性BMF 基因检测意义重大，但该试验技术难度较大，普遍开展有一定困难。下列临床建议可资参考：①对于＜3周岁患儿，诊断获得性AA 须非常慎重。②存在内脏畸形（如先天性心脏病、肾脏畸形等）者，需更多考虑先天性BFM 的可能性。故超声检查当为常规，但应避免为发现Fanconi贫血的骨骼畸形进行全身X 线检查。③起病急骤伴进展迅速者，多为获得性AA。故需尽量收集患儿以往血常规报告单，以分析可能的起病年龄与实际病程。

3.5.2　MDS 骨髓粒、红、巨核细胞系的病态造血是诊断MDS的最基本形态学基础。因红系病态造血及未成熟前体细胞异常定位等也可发生于AA，故不足以鉴别AA与MDS。1/3 的儿童MDS 由先天性BMF 转化而来，因此，一旦发现形态异常，应进行先天性BMF 基因检测、染色体核型及FISH检测、骨髓病理及免疫组织化学染色协助诊断。

3.5.3　原发性免疫性血小板减少症（immune thrombocytopenia，ITP）　AA 易被误诊为ITP，尤其是以胸骨穿刺检查结果为诊断依据时。AA 与ITP的骨髓巨核细胞数量和状态差异显著，故应严格执行近期我国《儿童ITP诊疗建议》将骨髓检查作为ITP 诊断主要条件之一的原则。在诊断ITP 时，应适时进行骨髓检查。

3.5.4　PNH　需要在避免近期输血的条件下检测CD^{55}和CD^{59}，以避免供血干扰检测结果。此外，PNH可能与AA 同时存在，或在病程中出现，故需要定期复查。

3.5.5　营养性贫血　如检查发现存在维生素B_{12} 或叶酸严重缺乏，需在营养补充纠正后再行复查。

3.5.6　各类溶血或自身免疫性疾病　均有原发疾病的特征性诊断依据，骨髓多呈明显增生，罕见骨

髓造血细胞明显减少者。

3.5.7 湿疹、血小板减少伴免疫缺陷综合征(wiskott-aldrich syndrome, WAS)除血小板减少之外,可伴有营养性贫血、继发溶血性贫血及严重感染。但WAS存在血小板体积明显缩小,骨髓巨核细胞可无明显减少,并具有严重湿疹和婴幼儿起病等特征,均可与获得性AA鉴别。

4 治疗

4.1 对症支持治疗 ①一般措施:避免剧烈活动,防止外伤及出血,尽量避免接触对骨髓造血有损伤作用的药物;注意饮食和口腔卫生;重型AA患者应予保护性隔离,有条件者应入住层流病房;避免出血,防止外伤及剧烈活动;必要的心理护理;可预防性应用抗真菌药物;欲进行移植及ATG/ALG治疗者建议给予预防性应用抗细菌、抗病毒及抗真菌治疗;造血干细胞移植后需预防卡氏肺孢子菌感染,如用复方磺胺甲恶唑,但ATG/ALG治疗者不必常规应用。②感染防治:SAA和VSAA患儿出现发热时,应按"中性粒细胞减少伴发热"的治疗处理。③成分血输注:根据卫生部2000年6月颁布的《临床输血技术规范》内科输血指南,红细胞输注指征为血红蛋白<60 g/L,但需氧量增加(如感染、发热、疼痛等)时可放宽红细胞输注指征。预防性血小板输注指征为血小板<10×10^9/L,存在血小板消耗危险因素者可放宽输注阈值。对严重出血者应积极给予成分血输注,使血红蛋白和血小板达到相对安全的水平。血小板输注无效者推荐HLA配型相合血小板输注。强调成分血输注,有条件时建议对血液制品进行过滤和/或照射。④造血生长因子的应用:对于粒细胞缺乏伴严重感染者可应用粒细胞集落刺激因子。⑤铁过载的治疗:对于反复输血所致铁过载,当血清铁蛋白>1000 g/L时可考虑祛铁治疗。⑥疫苗接种:推荐免疫抑制治疗(immunosuppressive therapy, IST)期间及停药半年内避免接种一切疫苗。停用IST半年后,如免疫功能大部分恢复或基本恢复可接种必要的灭活或减毒疫苗。

4.2 造血干细胞移植治疗 造血干细胞移植是治疗AA和先天性BMF的有效方法,具有起效快、疗效彻底、远期复发和克隆性疾病转化风险小等特点。移植时机与疾病严重程度、供体来源、HLA相合度密切相关,应严格掌握指征。

4.2.1 适应证 SAA、VSAA、输血依赖性NSAA或IST治疗无效的患者。

4.2.2 移植时机及供者的选择 SAA、VSAA患儿如有同胞相合供者,应尽快进行造血干细胞移植治疗;预计在短期(1~2个月)内能找到9~10/10位点相合的非血缘相关供者并完成供者体检的SAA、VSAA患儿,可在接受不包括ATG的IST治疗后直接进行造血干细胞移植;其余患儿在接受了包括ATG在内的IST治疗3~6个月无效后再接受造血干细胞移植治疗,应尽可能选择相合度高的非血缘或亲缘相关的供者进行移植。

4.2.3 造血干细胞的来源 骨髓、外周血及脐带血均可作为造血干细胞的来源。

4.2.4 注意事项 ①SAA和VSAA患儿一经确诊应尽早进行HLA配型。②输血依赖性NSAA的移植策略同SAA。③持续的粒细胞缺乏常使SAA,尤其是VSAA患儿面临难以控制的感染,但活动性感染并非移植的绝对禁忌证。而移植后粒细胞重建较快,通过移植重建的中性粒细胞来控制感染,可能是这些患儿生存的唯一希望。然而,由于这类移植的风险性高,因此,建议在具有相当移植经验的医院对患儿的疾病状态进行严格的评估,并在取得家长积极配合的前提下进行。④移植前需避免输注亲缘血液,尽量输注去除白细胞的血液制品以减少移植失败的几率。

4.3 IST IST是无合适供者获得性AA的有效治疗方法。目前常用方案包括抗胸腺/淋巴细胞球蛋白(antithymocyte /lymphocyte globulin, ATG / ALG)和环孢菌素A(cyclosporine, CsA)。

4.3.1 ATG/ALG

4.3.1.1 适应证 ①无HLA相合同胞供者的SAA和VSAA;②血象指标中有一项达SAA标准的NSAA和输血依赖的NSAA,且无HLA相合同胞供者;③第一次ATG/ALG治疗后3~6个月无效,且无合适供者行造血干细胞移植的患儿。ATG/ALG治疗应在无感染或感染控制后、血红蛋白80 g/L以上和血小板20×10^9/L以上时进行。

4.3.1.2 药物剂型与剂量 临床上ATG的应用相对比ALG更多,但疗效因动物来源和品牌的不同而存在差异。各种制剂的使用剂量及方法参见2014《儿童获得性再障的诊疗建议》。输注之前均应按照相应药品制剂说明进行皮试和/或静脉试验,试验阴性方可接受ATG/ALG治疗。每日用ATG/ALG时同步应用肾上腺糖皮质激素防止过敏反应。

4.3.1.3 不良反应和注意事项 ①ATG/ALG急性不

良反应包括超敏反应、发热、僵直、皮疹、高血压或低血压及液体潴留等，应给予泼尼松1～2 mg·kg^{-1}·d^{-1}或相应剂量其他糖皮质激素进行预防。患者床旁应备气管切开包、肾上腺素；②血清病：包括关节痛、肌痛、皮疹、轻度蛋白尿和血小板减少等，一般发生在ATG/ALG治疗后1周左右，糖皮质激素应足量应用至治疗后15 d，随后减量，一般2周减完（总疗程4周）。出现血清病反应者则静脉应用肾上腺糖皮质激素冲击治疗。第1次ATG/ALG治疗无效或复发患者2次治疗可选择HLA相合无关供者造血干细胞移植或第2次ATG/ALG治疗。选择第2次IST，与前次治疗应间隔3～6个月，第2个疗程的ATG/ALG，宜尽可能采用动物种属来源与前次不同的ATG/ALG剂型，以减少发生过敏反应和严重血清病风险。

4.3.2　CsA

4.3.2.1　适应证　①ATG/ALG治疗的SAA/VSAA患儿；②NSAA患儿。

4.3.2.2　使用方法　一旦确诊，应尽早治疗。口服起始剂量为5 mg·kg^{-1}·d^{-1}，由于儿童药物代谢快，CsA的应用剂量一般较成人大，剂量范围可以波动在5～10 mg·kg^{-1}·d^{-1}，所以测血药浓度尤为重要。服药2周后监测CsA血药浓度，建议全血谷浓度维持在100～150 μg/L，在保持谷浓度的前提下尽量将峰浓度维持在300～400 μg/L。疗效达平台期后12个月方可减量。应按原剂量的10%~20%递减，每3个月减量1次。减量期间密切观察血象，如有波动需慎重减量。一般CsA总疗程应在2～3年，减量过快可能增加复发风险。

4.3.2.3　不良反应与处理　主要不良反应为消化道症状、齿龈增生、色素沉着、肌肉震颤、肝肾功能损害，极少数患儿可发生头痛和血压增高，但大多症状轻微或对症处理后减轻，必要时可调换CsA剂型或选择其他免疫抑制剂。服药期间应定期监测血药浓度、肝肾功能和血压等。

4.3.3　其他IST　①大剂量环磷酰胺：由于大剂量环磷酰胺（45 mg·kg^{-1}·d^{-1}×4 d）的高致死率和严重毒性，不推荐其用于不进行造血干细胞移植的初诊患者或ATG/ALG联合CsA治疗失败的AA患者。②霉酚酸酯：对于该药的研究主要集中于治疗难治性AA，但多个中心研究表明MMF对难治性AA无效。③普乐可复：与CsA抑制T细胞活化的信号通路相同但作用更强、肾毒性更小，且无齿龈增生，因此被用来替换CsA用于AA的治疗，初步效果令人鼓舞，值得临床探索。④抗CD^{52}单抗：已有部分学者应用CD^{52}单抗治疗复发SAA，但仍缺乏大样本的临床研究来肯定该药物疗效，故目前仅推荐考虑作为二线方案，应用于治疗复发SAA。⑤雷帕霉素：在抑制T细胞免疫方面与CsA有协同作用，但最新研究显示，在ATG/ALG联合CsA基础上加用雷帕霉素不能提高患者的治疗反应率。雷帕霉素联合CsA治疗难治、复发AA的临床研究正在进行。

4.3.4　随访　接受ATG/ALG和CsA治疗的患者应密切随访，定期检查以便及时评价疗效和不良反应（包括演变为克隆性疾病如PNH、MDS和急性髓系白血病等）。建议随访观察点为ATG/ALG用药后3个月、6个月、9个月、1年、1.5年、2年、2.5年、3年、3.5年、4年、5年、10年。

4.4　刺激骨髓造血

4.4.1　TPO受体激动剂　TPO受体激动剂（也称为TPO类似物）可刺激骨髓中的巨核细胞及多能造血干细胞和祖细胞。其中艾曲波帕在美国FDA已批准用于难治性重型AA的治疗，近几年艾曲波帕联合IST在初治的SAA患者中也取得了良好疗效，但在中国仅批准用于12岁以上的患者。

4.4.2　G-CSF　没有证据表明G-CSF可纠正AA患者的基础造血干细胞缺陷，而且在免疫抑制治疗中加用G-CSF也并未改善患者的缓解率或生存情况。

4.4.3　雄激素　可以刺激骨髓红系造血，减轻女性患者月经期出血过多，是AA治疗的重要的辅助促造血用药物。其与CsA配伍，治疗非重型AA有一定疗效。一般应用司坦唑醇、十一酸睾酮或达那唑，应定期复查肝功能。

4.5　出现异常克隆AA患者的处理　少部分AA患者在诊断时存在细胞遗传学克隆异常，常见有+8、+6、13号染色体异常。一般异常克隆仅占总分裂象的很小部分，可能为一过性，可以自行消失。一些研究显示有无上述遗传学异常的AA患者对IST的反应类似。有异常核型的AA患者应该每隔3～6个月行1次骨髓细胞遗传学分析，异常分裂象增多提示疾病转化。

4.6　伴有明显PNH克隆的AA患者的处理　在AA患者中可检测到少量PNH克隆，患者骨髓细胞减少但并不出现溶血。通常仅单核细胞和中性粒细胞单独受累，并且仅占很小部分。推荐对这些患者的处理同无PNH克隆的AA患者。伴有明显PNH克

隆(>50%)及伴溶血临床及生化指标的AA患者慎用ATG/ALG治疗。AA-PNH或PNH-AA综合征患者的治疗以PNH为主,兼顾AA。推荐对于PNH克隆进行长期监测。

4.7 疗效标准 完全缓解:中性粒细胞绝对值>1.5×10^9/L,血红蛋白>110 g/L,血小板>100×10^9/L,脱离红细胞及血小板输注,并维持3个月以上。部分缓解:中性粒细胞绝对值>0.5×10^9/L,血红蛋白>80 g/L,血小板>20×10^9/L,脱离红细胞及血小板输注,并维持3个月以上。未缓解:未达到完全缓解或部分缓解标准。

5 转诊条件

5.1 从上级医院转诊到下级或基层医院 ①诊断明确及疾病状态稳定;②治疗方案确定且后续治疗可以在当地医院完成;③患儿生命体征平稳,临床没有重症感染及活动性出血表现。

5.2 从基层医院转诊到上级医院 ①诊断不明确及疾病状态不稳定,需要进一步的检查明确诊断和稳定病情;②当前治疗无法在基层医院完成;③基层医院无法处理的严重感染及出血状态。

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编写审定专家组
(按姓氏笔画排序)
组长:王天有
成员:马 洁 王天有 方拥军 刘玉峰
孙立荣 吴润晖 竺晓凡 金润铭
周 敏 郝国平 徐卫群

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Guidelines for the diagnosis and management of adult aplastic anaemia

Sally B. Killick, Writing Group Chair[1] Nick Bown,[2] Jamie Cavenagh,[3] Inderjeet Dokal,[4] Theodora Foukaneli,[5] Anita Hill,[6] Peter Hillmen,[6] Robin Ireland,[7] Austin Kulasekararaj,[7] Ghulam Mufti,[7] John A. Snowden,[8] Sujith Samarasinghe,[9] Anna Wood, BCSH Task Force Member[10] and Judith C. W. Marsh[7] on behalf of the British Society for Standards in Haematology

[1]The Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust, Bournemouth, [2]Northern Genetics Service, Newcastle upon Tyne, [3]St Bartholomew's Hospital, Barts Health NHS Trust, [4]Barts and The London School of Medicine and Dentistry, Queen Mary University of London and Barts Health NHS Trust, London, [5]Addenbrooks Hospital, University of Cambridge, Cambridge, [6]Leeds Teaching Hospitals, Leeds, [7]Kings College Hospital NHS Foundation Trust, London, [8]Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, [9]Great Ormond Street Hospital for Children NHS Foundation Trust, London, and [10]West Hertfordshire NHS Trust, Watford, UK

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Scope

Methodology

Literature review details. The guideline group was selected to be representative of UK-based aplastic anaemia (AA) medical experts. Recommendations are based on review of the literature using MEDLINE and PUBMED up to December 2014 under the heading: 'aplastic anemia'.

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria are specified in the BCSH guidance pack http://www.bcshguidelines.com/BCSH_PROCESS/EVIDENCE_LEVELS_AND_GRADES_OF_RECOMMENDATION/43_GRADE.html and the GRADE working group website http://www.gradeworkinggroup.org

The objective of this guideline is to provide healthcare professionals with clear guidance on the management of patients with AA. The guidance may not be appropriate to every patient and in all cases individual patient circumstances may dictate an alternative approach.

Working group membership. Review of the manuscript was performed by the British Committee for Standards in Haematology (BCSH) Haemato-Oncology Task Force, BCSH Executive Committee and then reviewed by a sounding board of the British Society for Haematology (BSH). This compromises 50 or more members of the BSH who have reviewed this guidance and commented on its content and applicability in the UK setting. It has also been reviewed by the Aplastic Anaemia Trust patient group but they do not necessarily approve or endorse the contents.

Correspondence: BCSH Secretary, British Society for Haematology, 100 White Lion Street, London N1 9PF, UK.
E-mail: bcsh@b-s-h.org.uk

Summary of key recommendations

Key recommendations for definition, severity and presentation

- **The severity of AA (AA) should be according to the Camitta criteria. Grade 1C**
- **Most cases of AA are idiopathic, nevertheless a careful drug history must be taken and any putative causative drug should be discontinued and reported to the Medicines and Healthcare products Regulatory Agency (MHRA) using the Yellow Card Scheme. Grade 1C**
- **A multidisciplinary team (MDT) meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should be given to seeking expert advice on the diagnosis and management of patients where there is uncertainty, or when an inherited bone marrow failure syndrome (IBMFS) is being considered.**

Key recommendations for inherited AA

- **Chromosomal breakage analysis of peripheral blood lymphocytes following exposure to diepoxybutane to test for Fanconi anaemia (FA) should be performed. Grade 1B**

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- **Comprehensive assessment should be performed, including family history, abdominal ultrasound, echocardiogram, high resolution computerized tomography scan of the chest and pulmonary function tests, and evaluation for other extra-haematopoietic abnormalities (such as cirrhosis, pulmonary fibrosis or renal anomalies); the presence of these will support a diagnosis of constitutional rather than idiopathic bone marrow failure (BMF). Grade 1B**

Key recommendations for supportive care

- **Blood transfusions should be given to improve quality of life. Grade 1A**
- **A threshold haemoglobin concentration cannot be recommended for all patients; it should be individualized according to co-morbidities. Grade 1A**
- **Phenotype (Rh and Kell) matched blood should be considered to reduce the risk of alloimmunization. Grade 1B**
- **Prophylactic platelet transfusions should be given to stable AA patients receiving active treatment. Grade 1B. A threshold (pre-transfusion) platelet count of 10×10^9/l should be used. Grade 1B**
- **In patients judged to have additional risk factors for bleeding, such as fever or sepsis, a higher prophylactic transfusion threshold of 20×10^9/l is recommended. Grade 2C**
- **Routine prophylactic platelet transfusions are not recommended for stable AA patients not on active treatment. Grade 2B**
- **Patients with chronic bleeding of World Health Organization grade 2 or above require individual management according to the severity of their symptoms and signs. Grade 2C**
- **Prior to administration of antithymocyte globulin (ATG), a daily threshold (pre-transfusion) platelet count of 20×10^9/l should be used for the duration of the ATG course. Grade 2C**
- **Only one adult platelet dose is routinely required. Grade 1A.**
- **All patients undergoing treatment with immunosuppressive therapy (ATG or Alemtuzumab) should receive irradiated blood products. Grade 1C**
- **All patients undergoing haemopoietic stem cell transplantation (HSCT) should receive irradiated blood products. Grade 1A**
- **The need for iron chelation therapy should be decided on an individual patient basis. Patients with iron overload after successful HSCT should undergo venesection. Grade 1B**
- **Aplastic anaemia patients who are severely neutropenic should be given prophylactic antibiotics and antifungal therapy according to local policies. Grade 2B**
- **Aplastic anaemia patients receiving immunosuppressive therapy (IST) should also receive prophylactic anti-viral agents, although routine prophylaxis against *Pneumocystis jirovecii* is not necessary. Grade 2C**

Key recommendations for IST

- **The current standard first line IST is horse ATG (ATG-ATGAM) combined with ciclosporin (CSA). Grade 1A**
- **Immunosuppressive therapy is recommended first line therapy for non-severe AA patients requiring treatment (see indications in text), severe or very severe AA patients who lack a matched sibling donor (MSD), and severe or very severe AA patients aged >35-50 years. Grade 1A**
- **A second course of ATG may be indicated following failure to respond to a first course [if the patient is ineligible for a matched unrelated donor (UD) HSCT] or following relapse after a first course. Grade 1A**
- **ATG is an immunosuppressive drug and should only be administered in centres familiar with its use; the drug must only be given to in-patients. Grade 1B**
- **The use of high dose or moderate dose cyclophosphamide (without stem cell support) is not recommended in AA. Grade 1A**
- **Following IST, vaccinations, including influenza, should be avoided if possible as there is a theoretical risk of disease relapse. Grade 2C**

Key recommendations for HSCT

- **All patients being considered for HSCT should be evaluated in a multi-disciplinary team setting, and consideration should be given to discussion of the case with a centre that has expertise in AA regarding the indications for HSCT and the choice of conditioning regimen. Grade 1C**
- **To inform the multi-disciplinary team decision-making regarding HSCT:**
  - **All patients who are potential HSCT candidates should undergo human leucocyte antigen (HLA) typing at diagnosis, followed by related or UD searches as appropriate to assess the availability of potential donors. Grade 1B**
  - **A careful reassessment should be made to confirm the precise diagnosis and exclude clonal evolution to myelodysplastic syndrome (MDS) or paroxysmal nocturnal haemoglobinuria (PNH), as this will influence the choice of conditioning. It is also vital not to miss constitutional AA so as to avoid (i) serious (and potentially lethal) toxicity from the transplant and (ii) inappropriate selection of a sibling donor. Grade 1C**
  - **The Haematopoietic Cell Transplant Co-morbidity Index or equivalent assessment should be documented. Grade 2B**

- **Alternatives to HSCT, including IST, should be actively considered in the management plan. Grade 1B**

- **Up-front MSD HSCT for young and adult patients is the treatment of choice for severe AA, but patients aged between 35-50 years need to be carefully assessed for co-morbidities prior to consideration for transplantation. Grade 1B**
- **Unrelated donor HSCT in adults should be considered after lack of response to one course of IST. Grade 1B**
- **There have been recent improvements in outcomes after alternative donor HSCT for patients who lack a suitably matched donor, but these transplants are still experimental and specialist advice should be sought; only European Bone Marrow Transplantation Severe Aplastic Anaemia Working Party (SAAWP) approved protocols should be used. Grade 2B**

Key recommendations for treatment of AA in the elderly

- **Elderly patients with AA should be individually assessed and their specific wishes respected, as quality of life is paramount in this patient group. Grade 1C**
- **Immunosuppressive therapy is considered the treatment of choice. ATG and CSA result in a more rapid recovery of blood counts but, alternatively, CSA alone or oxymetholone can be considered. Grade 1B**
- **Patients unfit for, who decline or who are intolerant of IST should be offered best supportive care. Grade 1C**
- **Eltrombopag is licensed by the European Medicines Agency (EMA) for severe AA refractory to IST or patients who are heavily pre-treated and unsuitable for HSCT. It should be used with meticulous long term monitoring for clonal evolution, or following a clinical research protocol. Grade 2B**

Key recommendations for management of AA in pregnancy

- **Supportive care remains the mainstay of treatment of AA in pregnancy, aiming to maintain the platelet count above 20×10^9/l with platelet transfusions. Grade 1C**
- **CSA is safe in pregnancy if needed. Grade 2C**

Key recommendations for PNH and AA

- **All patients should be screened for PNH using flow cytometry on peripheral blood to detect deficiency of glycosylphosphatidyl-inositol (GPI) anchored proteins, such as CD14, CD16 and CD24, as well as fluorescent aerolysin (FLAER) for white blood cells, and CD55 and CD59 for red cell analysis.**
- **Patients should be screened for PNH at the diagnosis of AA. If persistently negative, test 6-monthly for 2 years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes, positive, test 3-monthly for the first 2 years and only reduce the frequency if the proportion of the PNH cells has remained stable. Grade 2C**
- **Small PNH clones can be detected in up to 50% of patients with AA, usually without evidence of haemolysis; large clones are clinically significant and may result in haemolysis as well as increased thrombotic risk ('haemolytic PNH').**
- **Presence of a small/moderate PNH clone in AA does not directly influence the choice of treatment for the underlying BMF.**
- **New PNH patients should be referred to the PNH National Service to be monitored for PNH complications and assessed for anti-complement therapy.**

Definition, disease severity and clinical presentation of AA

Aplastic anaemia is a rare and heterogeneous disorder. It is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis. To diagnose AA there must be at least two of the following (Camitta *et al*, 1975) haemoglobin concentration (Hb) <100 g/l, platelet count $<50 \times 10^9$/l, neutrophil count $<1{\cdot}5 \times 10^9$/l. The majority (70-80%) of cases are idiopathic (Marsh *et al*, 2009). The remainder mainly consist of IBMFS. The incidence is 2-3 per million per year in Europe, but higher in East Asia (Montane *et al*, 2008). There is a biphasic distribution, with peaks at 10-25 years and over 60 years.

The modified Camitta criteria (Camitta *et al*, 1975; Bacigalupo *et al*, 1988) are used to assess severity:

- Severe AA (SAA);

  Marrow cellularity <25% (or 25–50% with <30% residual haematopoietic cells), plus at least 2 of: (i) neutrophils $<0{\cdot}5 \times 10^9$/l, (ii) platelets $<20 \times 10^9$/l (iii) reticulocyte count $<20 \times 10^9$/l (see diagnostic section for automated reticulocyte count)

- Very Severe AA (VSAA);

  As for SAA but neutrophils $<0{\cdot}2 \times 10^9$/l

- Non-severe AA (NSAA);

  AA not fulfilling the criteria for SAA or VSAA

Patients commonly present with symptoms of anaemia and thrombocytopenia. Serious infection is not a frequent symptom early in the course of the disease. A preceding history of jaundice may suggest a post-hepatitic AA. Whilst the majority of cases are idiopathic, a careful drug, occupational exposure and family history should be obtained. Any

putative drugs should be discontinued and the patient should not be re-challenged. If a possible drug association is suspected, this must be reported to the Medicines and MHRA using the Yellow Card Scheme (http://yellowcard.gov.uk). There is usually no hepatosplenomegaly or lymphadenopathy (except in infection). In young adults the presence of short stature, skin hyper/hypo pigmented areas and skeletal abnormalities, particularly affecting the thumb is suggestive of FA (Shimamura & Alter, 2010). The triad of nail dystrophy, reticular skin pigmentation and oral leucoplakia is characteristic of dyskeratosis congenita (DC) (Shimamura & Alter, 2010). The finding of peripheral lymphoedema may indicate a diagnosis of Emberger syndrome due to germline *GATA2* mutation.

Key recommendations for definition, severity and presentation

- **The severity of AA should be according to the Camitta criteria. Grade 1C**
- **Most cases of AA are idiopathic, nevertheless a careful drug history must be taken and any putative causative drug should be discontinued and reported to the MHRA using the Yellow Card Scheme. Grade 1C**
- **A MDT meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should be given to seeking expert advice on the diagnosis and management of patients where there is uncertainty, or when an IBMFS is being considered.**

Investigations required for the diagnosis of AA

Idiopathic AA is a diagnosis of exclusion and no single test reliably diagnoses idiopathic acquired AA. Consequently, the diagnostic evaluation must exclude assessment of alternative aetiologies of BMF. The "empty" marrow on histology of AA is characteristic and a prerequisite for the diagnosis. There is increasing recognition that IBMFS are commoner than previously thought and may present in adulthood. The following investigations (Table I) are required to confirm the diagnosis, and:

(i)exclude other causes of pancytopenia and a hypocellular bone marrow
(ii)exclude IBMFSs
(iii)screen for an underlying cause and
(iv)document co-existing abnormal cytogenetic and PNH clones.

See Table I for the summary of investigations for the diagnosis and further evaluation of AA; this table also summarizes the emerging diagnostics incorporating the latest molecular technologies that are likely to feature in the diagnosis and differential diagnosis within the next couple of years.

Both a bone marrow aspirate and trephine biopsy are required for the diagnosis of AA, and the key bone marrow findings are summarized in Table II.

The investigations in Table I should exclude non-AA causes of pancytopenia with a hypocellular bone marrow, which are listed in Table III.

A MDT meeting approach is recommended to collate relevant results and develop a treatment plan. Consideration should be given for seeking expert advice on the diagnosis and management of patients where there is uncertainty, or when an IBMFS is being considered.

Inherited AA

A number of inherited/genetic disorders are characterized by BMF/AA, usually in association with one or more somatic abnormality (Alter, 2007). The BMF typically presents in childhood but this can sometimes be in adulthood.

The two syndromes frequently associated with generalized BMF/AA are FA and DC (Dokal, 2011; Soulier, 2011), which can sometimes present with AA alone as their initial manifestation. These syndromes are genetically heterogeneous; 16 FA genes and 10 DC genes have been identified. The FA genes are important in DNA repair, the DC genes in telomere maintenance. Based on the DNA repair defect a diagnostic test-'chromosomal breakage test' is available for FA. Patients with DC usually have very short telomeres and this measurement [using flow cytometric fluorescence *in situ* hybridization or multiplex quantitative polymerase chain reaction (PCR)] can be useful in the assessment of DC. Genetic testing for known DC genes (representing *c.* 60% of cases) is possible in specialized centres.

In addition there are other genetic syndromes that are sometimes associated with AA/cytopenias. This includes Shwachman Diamond syndrome SDS (Dror *et al*, 2011) (mutations in *SBDS*), congenital amegakaryocytic thrombocytopenia CAMT (Ballmaier & Germeshausen, 2011) (mutations in *MPL*) and GATA2 deficiency (Emberger syndrome) (Horwitz, 2014) as well as genetically uncharacterized cases.

Some cases of inherited AA first present in adulthood and it is important to recognize these as their management differs from that of idiopathic AA. Where there are sufficient characteristic abnormalities a diagnosis may be straightforward (e.g. mucocutaneous features in DC). Where the presentation is only with AA and with minimal non-haematological abnormalities, inherited BMF should be considered and testing for known BMF syndromes should be undertaken. Investigations for inherited forms of AA should be re-appraised in patients initially classified as "idiopathic AA" and who fail to respond to anti-thymocyte globulin (ATG).

Key recommendations for inherited AA

- **Chromosomal breakage analysis of peripheral blood lymphocytes following exposure to diepoxybutane to test for FA should be performed. Grade 1B**

Table I. Summarized diagnosis and further investigation of aplastic anaemia.

Test	Key changes
1. Full blood count	Pancytopenia. Usually the haemoglobin concentration and neutrophil and platelet counts are uniformly depressed. In the early stages, isolated cytopenia, particularly thrombocytopenia, may occur. Lymphocyte counts are usually preserved. Presence of monocytopenia needs further investigation to exclude hairy cell leukaemia or inherited bone marrow failure due to *GATA2* mutation (Emberger/MonoMac syndrome, see section on inherited AA)
2. Reticulocyte count	Reticulocytopenia; automated reticulocyte counting will over-estimate the count compared with the levels set in the Camitta criteria (Camitta, 1984) for defining disease severity, which were defined on manual counts. This criterion has now been modified from manual percentages to absolute reticulocyte levels $<60 \times 10^9$/l as assessed by automated technologies (Rovo *et al*, 2013)
3. Blood film examination	Frequent macrocytosis and anisopoikilocytosis. Neutrophils may show toxic granulation. Platelets are mainly small in size. Exclude presence of dysplastic neutrophils, abnormal platelets, blasts and other abnormal cells, such as 'hairy' cells
4. HbF%	HbF; measure pre-transfusion in children – important prognostic factor in children. Note that the level is often elevated in constitutional syndromes
5. Peripheral blood chromosomal breakage analysis: diepoxybutane test (DEB Test)	For possible FA if patient aged <50 years, but it would also be indicated to screen older patients if FA is clinically suspected. It is difficult to set an upper age limit for FA screening, as anecdotal cases have been diagnosed in the fifth decade (unpublished observations). Screen all patients who are transplant candidates and siblings of FA patients
6. Flow cytometry for GPI-anchored proteins to detect PNH clone (6-colour methodology including FLAER)	See AA and PNH section for full description
8. Vitamin B12 and folate	Documented vitamin B12 or folate deficiency should be corrected before a final diagnosis of AA is confirmed. Bone marrow aplasia due to vitamin deficiency is exceedingly rare
9. Liver function tests	Liver function tests should be performed to detect antecedent/on-going hepatitis
10. Viral studies: hepatitis A/B/C, EBV, CMV, HIV and Parvovirus B19	AA due to hepatitis is rare, it usually occurs 2–3 months after an acute episode of hepatitis and is more common in young males (Brown *et al*, 1997). In post-hepatic AA the serology is often negative for the known hepatitis viruses. CMV should be assessed if SCT is being considered. HIV more commonly causes isolated cytopenias but is a very rare cause of AA (Wolf *et al*, 2007; Hapgood *et al*, 2013). Likewise, parvovirus B19 is more usually associated with pure red aplasia but has been reported with AA (Mishra *et al*, 2005)
11. Anti-nuclear antibody and anti-double stranded DNA	Pancytopenia in systemic lupus erythematosus may (i) be autoimmune with a cellular bone marrow (ii) associated with myelofibrosis or rarely (iii) with a hypocellular marrow
12. Chest X-ray and other radiology	Useful at presentation to exclude infection and for comparison with subsequent films. X-rays of the hands, forearms and feet may be indicated if an IBMFS is suspected. High resolution CT scan of the chest is indicated for suspected DC or constitutional *RUNX1* bone marrow failure syndrome
13. Abdominal ultrasound scan and echocardiogram	An enlarged spleen and/or lymph nodes raise the possibility of a malignant haematological disorder as the cause of the pancytopenia. In younger patients, abnormal or anatomically displaced kidneys are features of FA
14. Emerging diagnostic tests: the following are not currently routine diagnostic tests, but are likely to be so within the next few years	
Peripheral blood leucocyte telomere length:	Useful for disease screening for telomere gene mutations in classic DC; less specific in adult onset AA with *TERC*/*TERT* mutations; short telomeres may also occur in acquired AA with reduced stem cell reserve (Townsley *et al*, 2014)
Next generation sequencing, gene panels for:	• Telomere gene complex mutations • Other IBMFS • Acquired somatic mutations, typical of myeloid malignancies, to help distinguish AA from hypocellular MDS and for early detection of clonal evolution to MDS/AML (Kulasekararaj *et al*, 2014)
Single nucleotide polymorphism array karyotyping	Whole genome scanning to detect unbalanced chromosomal defects (Afable *et al*, 2011a)

HbF, fetal haemoglobin; GPI, glycerophosphatidylinositol; AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; FLAER, fluorescent aerolysin; EBV, Epstein Barr virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; SCT, stem cell transplantation; IBMFS, inherited bone marrow failure syndromes; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; CT, computerized tomography; DC, dyskeratosis congenita; FA, Fanconi anaemia.

Table II. Bone marrow features of aplastic anaemia.

Bone marrow aspirate	Can be performed without platelet support, providing adequate surface pressure is applied (Kelsey, 2003), even in severe thrombocytopenia. Difficulty obtaining fragments may indicate marrow fibrosis or infiltration and should raise the suspicion of a diagnosis other than AA. In AA, fragments and trails are hypocellular with prominent fat spaces and variable numbers of residual haemopoietic cells. Erythropoiesis is reduced or absent; dyserythropoiesis is very common, often marked and does not distinguish MDS from AA. Megakaryocytes and granulocytic cells are markedly reduced or absent. Dysplastic megakaryocytes and granulocytic cells are not seen in AA. Lymphocytes, macrophages, plasma cells and mast cells often appear prominent. In the early stages of disease, there may be increased macrophages with some haemophagocytosis and background eosinophilic staining representing interstitial oedema
Cytogenetic and FISH analysis	Karyotyping may fail in very hypocellular marrows with there being insufficient metaphases. In this situation perform FISH analysis for chromosomes 5, 7, 8 and 13 It was previously assumed that the presence of an abnormal cytogenetic clone indicated a diagnosis of MDS and not AA. However it is now evident that abnormal cytogenetic clones [such as del(13q), trisomy 8 and others], which may be transient, are present in up to 12% of patients with otherwise typical AA at diagnosis (Gupta *et al*, 2006; Afable *et al*, 2011b). Although monosomy 7 may indicate the likelihood of MDS in children, in adults monosomy 7 can also be seen in AA. Abnormal cytogenetic clones may arise during the course of the disease and the appearance of a new cytogenetic abnormality may provide evidence of clonal evolution (Maciejewski *et al*, 2002)
Bone marrow trephine biopsy	A good quality trephine biopsy of at least 2 cm is essential to assess overall cellularity and morphology of residual haemopoietic cells, and to exclude an abnormal infiltrate. Care should be taken to avoid tangential biopsies because subcortical marrow is normally hypocellular In most cases the biopsy specimen is hypocellular throughout; sometimes hypocellularity is patchy with both hypocellular and residual cellular areas. Focal hyperplasia of erythroid or granulocytic cells at a similar stage of maturation may be observed. Small lymphoid aggregates may occur, particularly in the acute phase of the disease or when AA is associated with systemic autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus. Increased reticulin staining, dysplastic megakaryocytes (best assessed by immunohistochemistry) and blasts are not seen in AA; their presence either indicates a hypoplastic MDS or evolution to leukaemia (Bennett & Orazi, 2009)

AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; FISH, fluorescence *in situ* hybridization; MDS, myelodysplastic syndrome.

- **Comprehensive assessment should be performed, including family history, abdominal ultrasound, echocardiogram, high resolution computerized tomography scan of the chest and pulmonary function tests, and evaluation for other extra-haematopoietic abnormalities (such as cirrhosis, pulmonary fibrosis or renal anomalies); the presence of these will support a diagnosis of constitutional rather than idiopathic BMF. Grade 1B**

Supportive care

Blood product support

Transfusion of red blood cells. For most patients with AA, transfusion with red blood cells (RBC) is essential to maintain a safe blood count, improve symptoms of anaemia and maintain quality of life. The decision to transfuse RBC should be based on clinical symptoms (signs of anaemia), taking into consideration the patient's age and co-morbidities (cardiac, pulmonary or vascular). Although no specific pre-transfusion haemoglobin concentration (Hb) trigger can be recommended, it is important to maintain quality of life and avoid symptoms. A higher trigger may be needed for elderly patients and those with co-morbidities. Optimal use of RBC transfusion involves administration of enough red cells to maximize clinical outcome whilst avoiding unnecessary transfusions (Carson *et al*, 2012).

Alloimmunization against red cell antigens and iron overload are the commonest risks associated with regular transfusion therapy. Provision of phenotype-matched blood (for Rh and Kell) should be considered to reduce the risk of alloimmunization.

Transfusion of platelets. Regular platelet transfusion support may be required for AA patients. With the exception of one publication (Sagmeister *et al*, 1999), literature specific to platelet transfusion support in AA is lacking, and evidence is taken from studies addressing the need for platelet transfusion support in patients with reversible thrombocytopenia (Estcourt *et al*, 2012; Stanworth *et al*, 2013; Killick *et al*, 2014). It is recommended that prophylactic platelet transfusions should be given to stable AA patients on active therapy (where the treatment aims to reverse the severe thrombocytopenia) with a platelet count $<10 \times 10^9$/l. For patients with sepsis, the platelet count should be kept $>20 \times 10^9$/l. For thrombocytopenic patients requiring invasive procedures, platelet transfusions must be administered, aiming to achieve a platelet count in line with BCSH guidelines for the relevant procedures (British Committee for Standards in Haematology, 2003), and a pre-procedure platelet count should be checked.

During treatment with ATG, worsening thrombocytopenia can occur. This is due to increased platelet consumption in the presence of cross-reacting antibodies in ATG binding to

Table III. Other causes of pancytopenia and a hypocellular bone marrow.

Associated with PNH (AA/PNH)	Variable cellularity depending on the phase of disease and transition from PNH to AA. Test peripheral blood immunophenotyping for GPI-linked molecules on red and white cell populations
Hypoplastic MDS/AML	Sometimes difficult to distinguish from AA. The following features of MDS are not found in AA: dysplastic cells of the granulocytic and megakaryocytic lineages, blasts in the blood, marrow aspirate or trephine biopsy specimen (Bennett & Orazi, 2009). In trephine biopsy specimens, increased reticulin, increased $CD34^+$ cells and residual areas of haemopoiesis suggests hypoplastic MDS rather than AA. The presence of ALIPs is more indicative of MDS than AA, though small collections of immature granulocytic cells may be seen in the bone marrow in AA when regeneration occurs. ALIPs must not be confused with dysplastic proerythroblast islands, and can be easily differentiated on immunohistochemistry. Dyserythropoiesis is very common in AA and does not distinguish MDS from AA
Hodgkin lymphoma or non-Hodgkin lymphoma	Can present with pancytopenia and a patchy hypocellular bone marrow with limited areas of lymphoid infiltration that can easily be missed in small samples. The bone marrow biopsy should be examined carefully for foci of lymphoma cells or fibrosis, which may be seen in only a small part of the specimen. Lymphocytes are often prominent in AA and immunophenotypic marker studies and gene rearrangement studies will help to exclude a diagnosis of lymphoma. Additional features, such as splenomegaly, make AA very unlikely
Primary myelofibrosis	Primary myelofibrosis is usually accompanied by abnormal blood film (teardrop poikilocytosis, leucoerythroblastic) changes and splenomegaly. The absence of an enlarged spleen in the presence of marrow fibrosis suggests a secondary malignancy
Mycobacterial infections	Sometimes present with pancytopenia and a hypocellular bone marrow. This is seen more commonly with atypical mycobacteria. Other bone marrow abnormalities include granulomas, fibrosis, marrow necrosis and haemophagocytosis. Demonstrable granulomas are often absent in *Mycobacterium tuberculosis* infection. AAFB are more frequently demonstrated in atypical mycobacterial infections where they are often phagocytosed by foamy macrophages. The bone marrow aspirate should be sent for AAFB and culture if tuberculosis is suspected (Bain *et al*, 2001)
Anorexia nervosa or prolonged starvation	May be associated with pancytopenia. The bone marrow may show hypocellularity, gelatinous transformation (serous degeneration/atrophy), loss of fat cells as well as haemopoietic cells, and increased background substance which stains a pale pink on haematoxylin/eosin stain (Bain *et al*, 2001). The pink background substance may also be seen on a May–Grünwald–Giemsa stained aspirate
ITP	Occasionally AA presents with an isolated thrombocytopenia, and pancytopenia develops later. Such patients can initially be misdiagnosed as ITP but bone marrow examination in AA shows hypocellularity with reduced or absent megakaryocytes, which is not commonly seen in ITP, although rarely ITP is associated with reduced megakaryocytes
AA in children	A recent comprehensive review discusses in more detail conditions that may present with pancytopenia and a hypocellular bone marrow in children (Davies & Guinan, 2007)
GATA2 deficiency – MonoMac	This diagnosis maybe considered in hypoplastic marrows with absent peripheral blood monocytes or severe monocytopenia (Spinner *et al*, 2014)

GPI, glycerophosphatidylinositol; AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; ALIPs, abnormal localization of immature precursors; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; AAFB, acid/alcohol fast bacilli; ITP, immune thrombocytopenia; MonoMac, monocytopenia with susceptibility to mycobacteria.

platelets. Although there are no studies to support the exact threshold for platelet transfusion support prior to ATG, most authors use a threshold of 20×10^9/l (Scheinberg *et al*, 2011; Scheinberg & Young, 2012).

Regular support with RBC and platelet transfusions increases the risk of HLA and non-HLA (minor histocompatibility) alloimmunization, leading to poor platelet increments and increased risk of graft rejection after HSCT. Leucodepletion of cellular blood components may reduce, but not eliminate, alloimmunization (Killick *et al*, 1997; Desmarets *et al*, 2009). The possibility of HLA alloimmunization and provision of HLA-selected platelets should be considered for patients refractory to platelet transfusion, provided other causes of refractoriness have been excluded. In the absence of HLA antibodies and for patients failing to increment with HLA-matched platelets, investigation and matching for human platelet antigen antibodies should be considered.

Granulocyte transfusions. The use of irradiated granulocytes should be considered in patients with life-threatening infection related to severe neutropenia (Quillen *et al*, 2009), and anecdotally may be life saving. Data about the effectiveness of granulocyte concentrates are limited and usage is linked with a number of adverse events, such as transfusion-related acute lung injury, alloimmunization and febrile reactions.

Use of irradiated cellular blood components for AA patients. Irradiation of cellular blood components prevents transfusion-associated graft-*versus*-host disease (TA-GVHD). This is a rare complication of blood transfusion with 100%

mortality. Irradiation may also reduce the risk of alloimmunization in AA, as reported from animal data (Bean *et al*, 1994).

- AA patients undergoing HSCT must be transfused with irradiated blood components in line with BCSH guidelines (Treleaven *et al*, 2011).
- All granulocyte concentrates and HLA-matched platelets must be irradiated.
- The risk of development of TA-GVHD following treatment with ATG, although appearing to be low, remains unclear. In view of the seriousness of the condition, and in line with previous BCSH guidelines and European Group for Blood and Marrow Transplantation (EBMT) recommendations (Marsh *et al*, 2010; Hochsmann *et al*, 2013), irradiated blood components are currently recommended for patients receiving ATG. It is not known how long the use of irradiated blood products following ATG treatment should be continued, but it may be reasonable to continue while patients are still taking CSA following ATG therapy.
- Patients treated with alemtuzumab must also receive irradiated blood components according to the BCSH guidelines (Treleaven *et al*, 2011).

CMV tested blood products. Following universal leucodepletion in the UK, the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) no longer recommends the use of cytomegalovirus (CMV)-negative blood components (if they have been leucodepleted) for patients with immunodeficiency (unless pregnant) and those undergoing HSCT (SaBTO Annual Report, 2011/12), although PCR monitoring should be considered (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/215126/dh_132966.pdf). To date, there has not been a statement from the British Society of Bone Marrow Transplantation regarding blood products and CMV status.

CMV-negative granulocyte components should be provided for CMV-negative recipients.

Iron chelation therapy. Aplastic anaemia patients on regular RBC transfusion support will develop tissue iron overload, but there remains debate on the clinical impact of transfusional iron overload. In the setting of HSCT, a raised serum ferritin is an adverse predictor of outcome in myeloablative stem cell transplantation (Armand *et al*, 2007). Although unreliable, serum ferritin remains the most widely quoted parameter for assessment of iron overload. Magnetic resonance imaging (T2* or R2) can quantitate cardiac and liver iron, and is a useful adjunct although its utility in AA has not been published.

There are few published data regarding iron chelation therapy in AA. A large study was the 1-year Evaluation of Patients' Iron Chelation with Exjade study (Lee *et al*, 2010). This confirmed that chelation with deferasirox can be administered safely in patients with AA (no drug-induced cytopenias were noted), and can reduce the serum ferritin. However, dose adjustments are required to adequately chelate those who are heavily transfusion dependent. Impaired renal function is observed with deferasirox, and the drug should be used with caution in AA patients who are taking CSA. Deferasirox is licensed for use in transfusion-dependent anaemia, but only as second line therapy when desferrioxamine is inadequate or contra-indicated. Deferiprone is efficacious but not recommended in neutropenic patients (Cermak *et al*, 2011).

For those responding to immunosuppression, or after a successful HSCT, venesection is recommended for iron overload.

Infection is the major cause of death in AA: prevention and treatment options

Infections remain the major cause of death in AA (Marsh & Kulasekararaj, 2013). In contrast to cancer patients undergoing chemotherapy, in SAA neutropenia is prolonged and persistent, resulting in a higher incidence of invasive fungal infection (IFI) and severe bacterial sepsis. Survival of non-responders to ATG in the last two decades has markedly improved and this has occurred in conjunction with decreased infection-related mortality and decreased frequency of IFIs (Valdez *et al*, 2011).

Prevention of infections. Aplastic anaemia patients who are severely neutropenic should ideally be nursed in isolation when in hospital. In the UK it is common practice to give prophylactic antibiotics and antifungals, regular mouth care including an antiseptic mouthwash (such as chlorhexidine or saline) and food of low bacterial content (Hochsmann *et al*, 2013). Prophylactic antibiotics, either two non-absorbables (e.g. colistin and neomycin) or quinolones (e.g. ciprofloxacin), may be initiated but the preference should be according to local policy. A mould (aspergillus) active azole, preferably itraconazole or posaconazole, should be used as prophylaxis. In the UK, prophylaxis against *Pneumocystis jirovecii* is not routinely given. Anti-viral prophylaxis in untreated patients with AA is not routinely given. Antiviral prophylaxis with aciclovir or valaciclovir should be used during and after ATG therapy. During ATG therapy, sub-clinical reactivation of CMV and Epstein–Barr virus (EBV) is common but self-limiting, and therefore does not need antiviral treatment; EBV-related post-transplant lymphoproliferative disease has only very rarely been reported after ATG, most often after rabbit ATG. It is not UK practice to give *Pneumocystis jirovecii* prophylaxis with ATG.

Treatment of infections. Protocols and guidelines for the management of febrile neutropenia, including the assessment and management of fungal infections, are well developed and clinicians should follow local hospital and National Institute for Health and Care Excellence guidance (Phillips *et al*, 2012). Empirical anti-fungal therapy, as per local guidelines,

should be initiated early for patients with clinically suspected IFIs, as these patients have persistent neutropenia. Granulocyte transfusions may be potentially life saving in severe sepsis, such as invasive fungal disease, particularly for patients due to proceed to HSCT (Quillen *et al*, 2009).

Haemopoietic growth factors. Haemopoietic growth factors, such as erythropoiesis-stimulating agents and granulocyte colony-stimulating factor (G-CSF), are usually ineffective in supporting blood counts in AA patients (Marsh *et al*, 2007), although encouraging preliminary results are reported with the thrombopoietin-mimetic, eltrombopag (Desmond *et al*, 2014); see also section on Treatment of AA in the Elderly.

Key recommendations for supportive care

- **Blood transfusions should be given to improve quality of life. Grade 1A**
- **A threshold haemoglobin concentration cannot be recommended for all patients; it should be individualized according to co-morbidities. Grade 1A**
- **Phenotype (Rh and Kell) matched blood should be considered to reduce the risk of alloimmunization. Grade 1B**
- **Prophylactic platelet transfusions should be given to stable AA patients receiving active treatment. Grade 1B. A threshold (pre-transfusion) platelet count of 10×10^9/l should be used. Grade 1B**
- **In patients judged to have additional risk factors for bleeding, such as fever or sepsis, a higher prophylactic transfusion threshold is recommended of 20×10^9/l. Grade 2C**
- **Routine prophylactic platelet transfusions are not recommended for stable AA patients not on active treatment. Grade 2B**
- **Patients with chronic bleeding of World Health Organization grade 2 or above require individual management according to the severity of their symptoms and signs. Grade 2C**
- **Prior to administration of ATG, a daily threshold (pre-transfusion) platelet count of 20×10^9/l should be used for the duration of the ATG course. Grade 2C**
- **Only one adult platelet dose is routinely required. Grade 1A.**
- **All patients undergoing treatment with IST (ATG or Alemtuzumab) should receive irradiated blood products. Grade 1C**
- **All patients undergoing HSCT should receive irradiated blood products. Grade 1A**
- **The need for iron chelation therapy should be decided on an individual patient basis. Patients with iron overload after a successful HSCT should undergo venesection. Grade 1B**
- **Aplastic anaemia patients who are severely neutropenic should be given prophylactic antibiotics and antifungal therapy according to local policies. Grade 2B**
- **Aplastic anaemia patients receiving IST should also receive prophylactic anti-viral agents, although routine prophylaxis against *Pneumocystis jirovecii* is not necessary. Grade 2C**

Immunosuppressive therapy

Current standard first line IST

Standard first line IST is the combination of horse ATG (ATGAM; Pfizer, New York, NY, USA) and CSA. Lymphoglobuline horse ATG is no longer available (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012). A prospective randomized study from the National Institutes of Health (NIH) and a prospective EBMT study showed significantly better response at 3 and 6 months, and survival with horse ATG compared to rabbit ATG for first line IST (Scheinberg *et al*, 2011; Marsh *et al*, 2012). There is no indication for routine use of G-CSF with ATG + CSA (Tichelli *et al*, 2011). Prednisolone is used with ATG for the sole purpose of prevention of side effects of ATG.

Indications

ATG + CSA is indicated as first line therapy for:

- NSAA patients who are transfusion dependent, bleeding, encountering infections or for lifestyle (activities).
- SAA)/VSAA patients in the absence of an HLA-matched sibling.
- SAA/VSAA patients >35-50 years of age (see Fig 1).

There is no upper age limit for ATG, but there is increased mortality in patients aged >60 years treated with ATG (Tichelli *et al*, 1999, 2011) (see later section on Treatment of AA in the Elderly). A second course of ATG may be indicated for failure to respond or relapse after a first course or if the patient is ineligible for UD HSCT (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012) (see Fig 2). For a second course, rabbit ATG may be given. A second course of horse ATG is an alternative option, but this may be associated with more immediate and late (serum sickness) side effects (Marsh *et al*, 2012). Compared to horse ATG, rabbit ATG produces more profound and prolonged lymphodepletion and, in some recent studies, more infections. It is therefore important to ensure that patients receive adequate prophylactic antimicrobial support when using rabbit ATG.

Administration of ATG

Antithymocyte globulin must be given as an in-patient. ATG is a powerful immunosuppressive agent; it should only be used in centres that are familiar with using the drug and with its side effects. Prior to starting ATG:

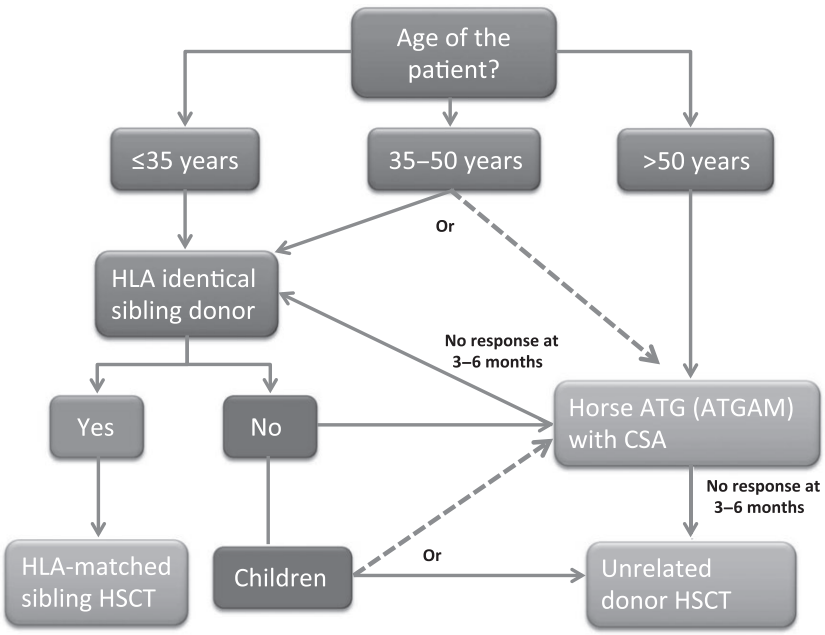

Fig 1. Treatment of acquired severe aplastic anaemia. HSCT may be considered, using a matched sibling donor or a suitably matched unrelated donor if no matched sibling donor is available, for patients aged 35–50 or >50 years who fail to respond to first line immunosuppressive therapy (Sureda *et al*, 2015). ATG, antithymocyte globulin; HLA, human leucocyte antigen; HSCT, haemopoietic stem cell transplantation; CSA, ciclopsporin.

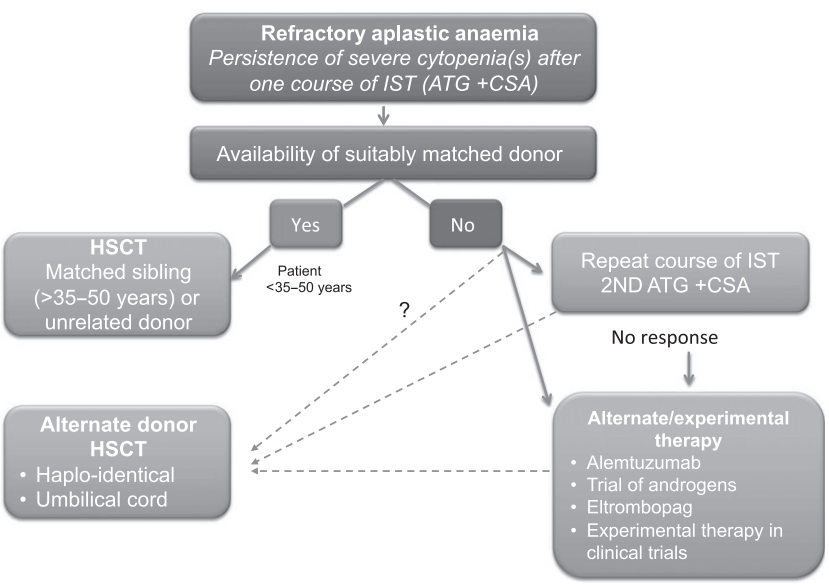

Fig 2. Treatment of adult refractory severe aplastic anaemia. ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplantation; IST, immunosuppressive therapy. Modified from Marsh, J.C. & Kulasekararaj, A.G. 2013.

- The patient should be clinically stable and ideally afebrile.
- Platelet count increment studies should be performed to exclude platelet refractoriness.
- Prophylactic antiviral, antibiotic and antifungal drugs should be administered according to local policy.
- For patients aged >60 years, careful assessment of co-morbidities is necessary to determine medical fitness prior to consideration for ATG, because there is increased mortality from infection and bleeding after ATG in this age group.

The dose of horse ATG (ATGAM) is 40 mg/kg/d for 4 d. It is given as an intravenous infusion over 12-18 h. Due to the risk of anaphylaxis, a 'test' dose must be given. Current practice is to use an intravenous infusion test dose (recent survey of the EBMT SAA Working Party, unpublished data May 2012), whereby the first 100 ml of the first day infusion is given over 1 h. ATG should be given through a double lumen Hickman or other central venous catheter, as it is sclerosing to peripheral veins, and also for ease of administration of other drugs and blood products. Each dose of ATG should be preceded with intravenous methyl prednisolone 1 mg/kg, chlorphenamine, and platelet transfusions aiming to keep the platelet count

>20–30 × 10^9/l (Marsh *et al*, 2009; Scheinberg & Young, 2012). Broad-spectrum intravenous antibiotics according to local policy should be given for febrile episodes irrespective of the neutrophil count. Fluid retention occurs commonly during ATG treatment, especially in older patients; careful attention to fluid balance is important. Prednisolone is started on the day after ATG is completed at a dose of 1 mg/kg/d for 2 weeks, followed by rapid tapering over the 2 weeks.

Ciclosporin should be commenced as the prednisolone dose is tapered, at a dose of 5 mg/kg/d to achieve trough blood levels of 100–200 μg/l. CSA should be continued whilst the blood count continues to rise. A slow tapering of the drug (25 mg every 2 3 months) can be started after at least a further 12 months of therapy, to reduce the risk of later relapse (Dufour *et al*, 2013).

Side effects of ATG are (i) early reactions, including fever, rash, rigors, hypo/hypertension, fluid retention, rarely acute pulmonary oedema/adult respiratory distress syndrome and anaphylaxis and (ii) later, serum sickness occurring days 7 14 from the start of ATG, most commonly with arthralgia, myalgia, rash and fever.

Serum sickness is treated with intravenous hydrocortisone 100 mg four times a day (QDS) and adequate analgesia; it usually requires a few days of treatment. Extra platelet transfusions are often needed during the period of serum sickness due to platelet consumption.

There is no indication for using G-CSF with ATG + CSA, as prospective randomized studies have shown that daily G-CSF given for 3 months after ATG does not improve response or overall survival (OS) (Tichelli *et al*, 2011).

Table IV. Criteria for response to IST in AA (Marsh *et al*, 2009).

(a) Response criteria following IST in severe AA	
None	Still fulfil severe disease criteria
Partial	Transfusion independent No longer meet criteria for severe disease
Complete	Haemoglobin concentration normal for age and gender Neutrophil count >1·5 × 10^9/l Platelet count >150 × 10^9/l
(b) Response criteria following IST for non-severe AA	
None	Blood counts are worse, or do not meet criteria below
Partial	Transfusion independence (if previously dependent) *or* doubling or normalization of at least one cell line *or* increase of baseline • haemoglobin concentration of >30 g/l (if initially <60) • neutrophils of >0·5 × 10^9/l (if initially <0·5) • platelets of >20 × 10^9/l (if initially <20)
Complete	Same criteria as for severe disease

AA, aplastic anaemia; IST, immunosuppressive therapy.

Outcomes

Response to ATG (as defined in Table IVa,b) is delayed, starting after an average of 3 4 months. The 6-month response rate to a first course of horse ATG is around 70%. Five-year OS is age-dependent: 100% for age <20 years, 92% for 20–40 years, 71% for 40–60 years and 56% for >60 years (Tichelli *et al*, 2011). In comparison, the response to a first course of rabbit ATG is around only 35 45%, with significantly worse OS (Scheinberg *et al*, 2011; Marsh *et al*, 2012; Scheinberg & Young, 2012). For NSAA, ATG + CSA results in significantly higher response rates, 74% *versus* 46% (and better event-free survival), compared to CSA alone (Marsh *et al*, 1999). Relapse after ATG occurs in up to 35% of patients; the risk of later clonal evolution to MDS/acute myeloid leukaemia is 15%, and haemolytic PNH in 10% (Rosenfeld *et al*, 2003; Scheinberg & Young, 2012).

Response to a second course of ATG from most studies is around 35% for refractory AA and 55–60% for relapsed AA (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012). Factors predicting for response are summarized in Table V.

Other immune suppressive drugs that have been used in AA

It is recommended that expert advice be sought when considering the use of other immunosuppressive drugs. Mycophenolate mofetil, sirolimus, corticosteroids and

Table V. Factors predicting response to ATG.

1	Young age
2	Less severe disease
3	Absolute reticulocyte count >25 × 10^9/l and absolute lymphocyte count >1·0 × 10^9/l (Scheinberg *et al*, 2009)
4	The finding of either of the chromosomal abnormalities trisomy 8 or del(13q) in the context of AA predicts for good response to ATG (Maciejewski *et al*, 2002; Holbro *et al*, 2013)
5	The presence of a PNH clone is predictive of response in some but not all studies
6	Telomere length is not predictive of response, but longer telomeres identify a sub-group who show excellent overall survival after IST (Scheinberg *et al*, 2010)
7	Response to a second course of ATG from most studies is around 35% for refractory AA and 55–60% for relapsed AA (Marsh *et al*, 2009; Passweg & Marsh, 2010; Scheinberg & Young, 2012)

AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; ATG, antithymocyte globulin; IST, immunosuppressive therapy.

Table VI. Other immunosuppressive drugs that have been used in AA.

Alemtuzumab	Effective in around 35% and 55% of patients with refractory and relapsed AA, respectively Not recommended as first line IST, as a response rate of only 19% was reported from the prospective NIH study (Scheinberg *et al*, 2012). May be considered as an option for refractory/relapsed AA (i) when a second course of ATG is not possible, or (ii) in the presence of renal impairment, as it is effective as monotherapy without addition of CSA or (iii) if the patient is ineligible for HSCT Given as a total dose of 100 mg; given as a subcutaneous dose of 10, 30, 30 and 30 mg over 4 d. Relapses are frequent although patients may respond again to a further course. All patients should receive adequate prophylaxis including against *Pneumocystis jirovecii* Patients being considered for alemtuzumab should be referred to a tertiary centre, be treated as part of the established EBMT protocol and reported to EBMT registry
Mycophenolate mofetil and sirolimus	There is no indication for the addition of other immunosuppressive drugs, such as mycophenolate mofetil or sirolimus, either in addition to ATG or in isolation, as there is no evidence that they are effective in AA In combination with ATG + CSA they do not increase the response rate, survival or reduce relapse, compared to ATG + CSA (Scheinberg & Young, 2012)
Cyclophosphamide	The use of high dose or even so called 'moderate' dose cyclophosphamide as treatment for AA is not recommended. Although response occurs in around 50% of patients with refractory AA, its predictable prolonged duration of neutropenia results in a high incidence of severe fungal infections and mortality (Tisdale *et al*, 2000; Marsh *et al*, 2009; Samarasinghe & Webb, 2012; Scheinberg & Young, 2012; Scheinberg *et al*, 2014)

AA, aplastic anaemia; IST, immunosuppressive therapy; ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplantation; EBMT, European Group for Bone Marrow Transplantation.

cyclophosphamide are not recommended in the treatment of AA (see Table VI).

Vaccinations in non-transplanted patients

There is a potential relapse risk of AA following vaccinations in those patients who have responded to IST. The evidence base is limited and based on anecdotal case reports, as well as an appreciation that a viral insult is likely to be an important trigger in the pathogenesis of AA (Viallard *et al*, 2000; Hendry *et al*, 2002). Vaccinations, including influenza vaccination, should be avoided if possible, except following HSCT, when AA patients should be routinely vaccinated as recommended for all allogeneic bone marrow transplantation recipients (see HSCT section).

Key recommendations for IST

- **The current standard first line IST is horse ATG (ATG-ATGAM) combined with CSA. Grade 1A**
- **Immunosuppressive therapy is recommended first line therapy for non-severe AA patients requiring treatment (see indications in text), severe or very severe AA patients who lack a MSD, and severe or very severe AA patients aged >35-50 years. Grade 1A**
- **A second course of ATG may be indicated following failure to respond to a first course (if the patient is ineligible for a matched UD HSCT) or following relapse after a first course. Grade 1A**
- **ATG is an immunosuppressive drug and should only be administered in centres familiar with its use; the drug must only be given to in-patients. Grade 1B**
- **The use of high dose or moderate dose cyclophosphamide (without stem cell support) is not recommended in AA. Grade 1A**
- **Following IST, vaccinations, including influenza, should be avoided if possible as there is a theoretical risk of disease relapse. Grade 2C**

Haemopoietic stem cell transplant in AA

Current indications for HSCT in adults

The current indications for HSCT are based on the EBMT SAAWP guidelines (Sureda *et al*, 2015). Patients should be managed in JACIE [Joint Accreditation Committee-International Society for Cellular Therapy (ISCT) and EBMT]-accredited centres.

HLA identical sibling donor. Up-front HSCT from a MSD is indicated for SAA in young and adult patients who have a MSD. EBMT data show similar outcomes for patients aged 40-50 to those aged 30-40 years (Sureda *et al*, 2015). However, co-morbidities should be carefully assessed to determine fitness for up-front transplantation instead of IST for patients aged 35-50 years.

Unrelated donor. Unrelated donor HSCT is indicated for SAA after failure to respond to one course of IST. There is no strict upper age limit but this should be discussed on an individual patient basis and according to co-morbidities at the respective transplant centre. The donor should be 10/10- or 9/10-matched based on HLA high resolution typing for class I (HLA-A, -B, -C) and II (HLA-DRB1, -DQB1) antigens.

Alternative donor: cord blood and haploidentical. Alternative donor HSCT using either cord blood, a haploidentical family donor or a 9/10-matched UD may be considered, among other treatment options, after failure to respond to IST and in the absence of a MSD and a suitably matched UD (Samarasinghe *et al*, 2012; Passweg & Aljurf, 2013). All donors should be screened for donor-directed HLA antibodies, the presence of which is associated with a very high risk of graft rejection. There is less clear guidance on the exact indication for alternative donor HSCT as this is less successful than MSD or UD HSCT, but new approaches to alternative donor HSCT are being evaluated using uniform EBMT protocols.

Syngeneic donor. In the rare situation where there is a syngeneic donor available, HSCT should be considered in all patients regardless of age as long term OS exceeds 90% (Marsh & Kulasekararaj, 2013).

Timing of donor search/availability

For all newly diagnosed AA patients who may be potential transplant candidates, HLA tissue typing should be performed at time of diagnosis, so that (i) MSD HSCT can proceed as soon as possible, and ideally before the patient becomes sensitized, not only to HLA but also to minor histocompatibility antigens, and (ii) the potential availability of UDs is established, so that if there is no response to a course of ATG and CSA, the patient can then proceed to UD HSCT (or earlier if the patient's condition is of concern with severe and/or recurrent infections). Assessment for response to IST is usually made at 3 6 months.

Pre-transplant work up

An MDT approach is essential for the pre-transplant work up. The aims of the work up are to (i) confirm the diagnosis and exclude/document clonal evolution (ii) assess co-morbidities (iii) select the donor, conditioning regimen, stem cell dose and source, (iv) address fertility issues and (v) inform the transfusion laboratory of the potential transplant and review of transfusion requirements (Table VII).

Conditioning regimens

The choice of conditioning regimens to use depends on (i) patient age (ii) type of donor (iii) centre preference for choice of antibody, whether ATG (Bacigalupo *et al*, 2010; Sanders *et al*, 2011) or alemtuzumab (Marsh *et al*, 2011; Bacigalupo *et al*, 2012). See Table VIII.

How successful is HSCT for AA?

For adult MSD HSCT, the survival is age-dependent, but OS is 70-85% between the ages of 30 and 50 years. A recent EBMT analysis has shown that outcomes after UD HSCT are no longer inferior to MSD HSCT, in that UD is not a negative predictor of survival (Bacigalupo *et al*, 2013; Marsh *et al*, 2014).

Specific issues relating to AA HSCT regarding early post-transplant management and management of late effects are summarized in Table IX.

Key recommendations for haemopoietic stem cell transplantation

- **All patients being considered for HSCT should be evaluated in a multi-disciplinary team setting, and consideration should be given to discussion of the case with a centre that has expertise in AA regarding the indications for HSCT and the choice of conditioning regimen. Grade 1C**
- **To inform the multi-disciplinary team decision making regarding HSCT:**
  - **All patients who are potential HSCT candidates should undergo HLA typing at diagnosis, followed by related or UD searches as appropriate to assess the availability of potential donors. Grade 1B**
  - **A careful reassessment should be made to confirm the precise diagnosis and exclude clonal evolution to MDS or PNH, as this will influence the choice of conditioning. It is also vital not to miss constitutional AA so as to avoid (i) serious (and potentially lethal) toxicity from the transplant and (ii) inappropriate selection of a sibling donor. Grade 1C**
  - **The Haematopoietic Cell Transplant Co-morbidity Index or equivalent assessment should be documented. Grade 2B**
  - **Alternatives to HSCT, including IST, should be actively considered in the management plan. Grade 1B**
- **Up-front MSD HSCT for young and adult patients is the treatment of choice for severe AA, but patients aged between 35 50 years need to be carefully assessed for co-morbidities prior to consideration for transplantation. Grade 1B**
- **Unrelated donor HSCT in adults should be considered after lack of response to one course of IST. Grade 1B**
- **There have been recent improvements in outcomes after alternative donor HSCT for patients who lack a suitably matched donor, but these transplants are still experimental and specialist advice should be sought; only European Bone Marrow Transplantation SAAWP approved protocols should be used. Grade 2B**

Treatment of AA in the elderly

The treatment of elderly patients (aged >60 years) with AA is more complex than in younger patients. In addition, the

Table VII. Pre-transplant work up.

Confirm diagnosis and exclude/document clonal evolution	Perform a reassessment BM aspirate, trephine biopsy, cytogenetic analysis (and FISH for chromosomes 5, 7, 8 and13 if cytogenetic analysis fails) to confirm the diagnosis is still AA, and to exclude other causes of pancytopenia, such as hypocellular MDS (see diagnostic section)
	Repeat flow cytometry to document whether there is a PNH clone
	Exclude a constitutional form of AA (see diagnostic section, emerging diagnostics), for example FA or DC not only in children but also adults. Late onset FA or DC may present without the classical somatic abnormalities, and instead may be associated with, for example, pulmonary fibrosis or cirrhosis, which may both impact on transplant outcomes (Gerull *et al*, 2013). Conditioning regimens are different from those used in acquired AA, which are likely to be fatal in undiagnosed constitutional AA. Avoid using a MSD with an unsuspected constitutional AA
	Consider referral for opinion/advice to a centre with AA expertise and access to integrated diagnostic laboratories, including molecular genetic techniques to help differentiate AA from MDS and to exclude constitutional AA
Assess co-morbidities	Follow standard guidelines as for all patients undergoing allogeneic HSCT and document the Hematopoietic Cell Transplant Co-morbidity Index or equivalent
	As AA patients are likely to be multi-transfused at the time of HSCT, assess for iron overload with serum ferritin and, if available, T2* MRI scan for assessment of cardiac and liver iron can be considered (see section Blood Product Support for patients with AA)
	Perform serum HLA antibody screen to assess for HLA antibodies. This is to (i) ensure adequate platelet count increments and (ii) select the appropriate donor for patients being considered for mis-matched HSCT, whether using cord blood, haploidentical or a 9/10-matched unrelated donor
Select donor, conditioning regimen, stem cell source and dose	Choice of donor and type of conditioning regimen is usually straightforward but not always, so consider discussion with a centre with AA expertise
	Compared to HSCT for haematological malignancies, a higher stem cell dose is required, in order to reduce the risk of graft failure. For MSD and UD HSCT, a minimum of 3×10^6 CD34-positive cells/kg (or 3×10^8 TNC/kg) is required. For cord blood HSCT, a minimum of 4×10^7 TNC/kg is recommended, thus usually necessitating a double cord infusion (Passweg & Aljurf, 2013). There is no consensus on cell dose for haploidentical HSCT, but a proposed algorithm for donor selection to optimize the cell dose includes using, if possible, a young and male family donor (Parikh & Bessler, 2012)
	For ATG-based conditioning regimens, BM is the preferred stem cell source (http://ebmtonline.forumservice.net; Bacigalupo *et al*, 2010). For alemtuzumab-based regimens, either BM or PBSC may be used. The use of PBSC to increase the stem cell dose is being explored in the EBMT SAAWP protocol for haploidentical HSCT (Clay *et al*, 2014)
Address fertility issues	AA patients receiving high dose cyclophosphamide as part of the conditioning regimen are likely to retain their fertility post-HSCT (Ciurea & Champlin, 2013). Less long term data are available using fludarabine with lower dose cyclophosphamide regimens, although cases of successful pregnancy have been reported. The effect of low dose TBI (2 Gy) is another factor
	For patients of childbearing age, referral to an assisted conception unit for discussions on fertility should be offered. Men should be offered sperm storage. Women should have the opportunity to discuss with an assisted conception unit specialist the latest results of egg/embryo cryopreservation so they can decide if they wish to proceed with this
	However, if the patient has on-going systemic sepsis and needs an urgent HSCT, the procedure of gonadal hyperstimulation may be too dangerous. In addition, in the presence of a significant PNH clone, the risk of venous thrombosis is further increased by the state of gonadal hyperstimulation, and in this situation expert advice from one of the two national UK PNH centres should be sought regarding the use of eculizumab

AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; IBMFS, inherited bone marrow failure syndrome; MDS, myelodysplastic syndrome; ATG, antithymocyte globulin; CSA, ciclosporin; FA, Fanconi anaemia; DC, dyskeratosis congenital; HSCT, haemopoietic stem cell transplantation; MSD, matched sibling donor; UD, unrelated donor; EBMT, European Group for Bone Marrow Transplantation; SAAWP, Severe Aplastic Anaemia Working Party; TBI, total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; MRI, magnetic resonance imaging; HLA, human leucocyte antigen; TNC, total nucleated cells.

outcome is worse due to inferior tolerability of the treatment. Therefore patients should be individually assessed for co-morbidities and their specific wishes should be respected, as quality of life is an important outcome in this group. With regard to diagnosis, it is important to exclude hypoplastic MDS, as MDS is far more common than AA in this age group (see diagnostic section).

Older age *per se*, is not a reason to withhold treatment even in the very elderly. Immunosuppression is considered the treatment of choice. There is no place for allogeneic HSCT as first line therapy in patients aged >60 years, although HSCT can be considered in selected patients with a syngeneic donor. Ideally, the least toxic and most convenient treatment should be given. However, another consideration

Table VIII. Conditioning regimens used in HSCT for severe AA.

Matched sibling donor	For patients aged <30 years, high dose CY (200 mg/kg) with ATG or alemtuzumab. Post-graft immune suppression with CSA and 'short' course MTX if using ATG, or CSA alone if using alemtuzumab For patients aged >30 years, fludarabine 30 mg/m^2 × 4, CY 300 mg/m^2 × 4 and ATG ('FCATG') or alemtuzumab ('FCC'). Post-graft immune suppression as for patients aged <30 years Post-graft CSA is usually continued for 9 months with tapering of dose over 3 months, to reduce late graft failure There is no indication for using radiation as part of the conditioning regimen
Unrelated donor	For 10/10-matched UD HSCT, for adults, the choice is either (i) the EBMT protocol of FCATG with 2 Gy TBI or (ii) FCC without TBI For 9/10-matched UD HSCT, either FCATG + 2 Gy TBI or FCC + 2 Gy TBI
Cord blood	There is no consensus but it is recommended that the EBMT-adopted French protocol be followed, using fludarabine, CY 120 mg/kg, ATG, TBI 2 Gy, with one dose of rituximab on day +5, total nucleated cell dose infused >4 × 10^7/kg and not less than 4 out of 6 HLA mis-matched cord units (Passweg & Aljurf, 2013)
Haploidentical family	There is no consensus (Passweg & Aljurf, 2013) but it is recommended that the current EBMT SAAWP protocol be followed, using non-myeloablative conditioning (CY 14·5 mg/kg × 2, fludarabine 30 mg/m^2 × 4, TBI 2 Gy) with post-graft high dose CY (50 mg/kg on days +3 and +4) with tacrolimus and MMF post-graft. Either BM or PBSC can be used, but a high stem cell dose is essential (Clay *et al*, 2014)
Syngeneic	Conditioning prior to stem cell infusion is recommended, using high dose CY and probably also ATG. There may be good rational for using PBSC in preference to BM, as the use of PBSC is associated with a lower risk of graft failure in the setting of syngeneic HSCT (Marsh & Kulasekararaj, 2013)

AA, aplastic anaemia; ATG, antithymocyte globulin; CSA, ciclosporin; HSCT, haemopoietic stem cell transplant; MSD, matched sibling donor; UD, unrelated donor; CY, cyclophosphamide; MTX, methotrexate; FCC, fludarabine, cyclophosphamide, alemtuzumab (Campath); EBMT, European Group for Bone Marrow Transplantation; SAAWP, Severe Aplastic Anaemia Working Party; TBI, total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; MMF, mycophenolate mofetil.

Table IX. Management of early issues and late complications post-HSCT for severe AA.

Early post-transplant management	• Post-graft CSA is continued for 9 months followed by tapering to 12 months, to reduce the risk of late graft failure • Blood CSA trough levels need to be maintained at higher levels than used in haematological malignancies, between 300 and 350 μg/l. If renal function is compromised, a 'half dose' CSA and 'half dose' MMF regimen can be used instead • Regular monitoring of unfractionated and lineage-specific CD3 (T-cell) chimaerism in peripheral blood and bone marrow is recommended to detect early graft failure. Progressive mixed chimaerism predicts a high risk of graft rejection. Stable mixed T-cell chimaerism in the presence of full donor myeloid chimaerism is common when using the FCC regimen (Marsh *et al*, 2011; Bacigalupo *et al*, 2012)
Management of late effects	• Late effects monitoring should follow international guidelines, and these include routine surveillance for secondary malignancy, endocrine, metabolic, bone (including avascular necrosis) and cardiovascular risks (Majhail *et al*, 2012) • The risk of second malignancy in AA HSCT is reduced by avoiding irradiation and by the absence of chronic GVHD • Iron overload is common and is most easily addressed by regular venesections once patients are fully engrafted post-transplant • In transplanted AA patients, re-vaccination should proceed as per standard allogeneic HSCT practice

AA, aplastic anaemia; ATG, antithymocyte globulin; CSA, ciclosporin; MMF, mycophenolate mofetil; HSCT, haemopoietic stem cell transplantation; FCC, fludarabine, cyclophosphamide, alemtuzumab (Campath); GVHD, graft-*versus*-host disease.

is how quickly a response is required, such that those with life threatening cytopenias (neutrophil count <0·2 × 10^9/l) or having suffered a severe infection requiring hospitalization should be treated more intensely than those with less severe disease.

Treatment with ATG and CSA results in a more rapid and complete response than CSA alone in patients with NSAA (Marsh *et al*, 1999). However, patients require hospitalization and have a higher risk of acute and delayed toxicity than younger patients, so the risks and benefits of treatment should be weighed up for each individual patient. Patients must be assessed carefully before treatment, as the risk of infection, bleeding, heart failure and arrhythmias with ATG is higher in the elderly. Older patients have an inferior survival after ATG compared to younger patients (Tichelli *et al*, 1999).

Alternative treatments include CSA alone, oxymetholone (or danazol) or alemtuzumab. Although the response rate of CSA alone is inferior to the combination of ATG and CSA in NSAA, OS is not inferior as CSA-refractory patients may

respond to second line therapy with ATG and CSA (Marsh *et al*, 1999). CSA alone has the convenience of being outpatient-based but patients must be carefully monitored for nephrotoxicity and hypertension. Alemtuzumab may be used as a single agent in refractory/relapsed AA, but medical fitness needs very careful assessment in older patients prior to considering this agent as a possible option (Scheinberg *et al*, 2012).

Oxymetholone or danazol can be considered in men intolerant or unresponsive to CSA (Allen *et al*, 1968; Jaime-Perez *et al*, 2011). Danazol has fewer masculinizing side effects than oxymetholone so may be a better alternative for women. Careful monitoring of oxymetholone is required as it can cause nephrotoxicity, hepatic tumours, mood changes, cardiac failure, prostatic enlargement and raised blood lipids.

Patients who are intolerant of, or who decline IST should be offered best supportive care.

Eltrombopag

Eltrombopag is a peptide, small molecule, oral thrombopoietin receptor agonist. In an extension of an earlier phase II study at NIH, 43 patients with refractory SAA were treated with eltrombopag (Desmond *et al*, 2014). Haematological responses, including trilineage response, were observed in 40% of patients. The drug was well tolerated in most patients. Elevated transaminase levels may occur and there are particular concerns about clonal evolution, including monosomy 7, which requires further evaluation. Eltrombopag has been approved by the Food and Drug Administration in the USA for treatment of SAA refractory to IST. It has recently, as of August 2015, been licensed by the EMA for SAA refractory to IST or patients who are heavily pretreated and unsuitable for HSCT. It should be used with meticulous long term monitoring for clonal evolution, or following a clinical research protocol. It is advised that a repeat bone marrow is performed prior to starting treatment to exclude an abnormal cytogenetic clone typical of MDS/AA, particularly monosomy 7.

Key recommendations for treatment of AA in the elderly

- **Elderly patients with AA should be individually assessed and their specific wishes respected, as quality of life is paramount in this patient group. Grade 1C**
- **Immunosuppressive therapy is considered the treatment of choice. ATG and CSA result in a more rapid recovery of blood counts but, alternatively, CSA alone or oxymetholone can be considered. Grade 1B**
- **Patients unfit for, who decline or who are intolerant of IST should be offered best supportive care. Grade 1C**
- **Eltrombopag is licensed by the EMA for SAA refractory to IST or patients who are heavily pre-treated and unsuitable for HSCT. It should be used with meticulous long term monitoring for clonal evolution, or following a clinical research protocol. Grade 2B**

Management of AA in pregnancy

Although the relationship, either casual or coincidental, between AA and pregnancy is controversial, it remains a serious condition, challenging to manage and with a variable clinical outcome. AA can be diagnosed for the first time during pregnancy, in early or late gestation. Cytopenia(s) often progresses during pregnancy, but the disease may remit spontaneously, after abortion (spontaneous or therapeutic) or after delivery (Aitchison *et al*, 1989). Relapse is common during pregnancy in AA patients who have previously responded to ATG, especially those with partial response (Tichelli *et al*, 2002). Pregnancy does not trigger relapse of the disease in patients who had undergone successful HSCT.

Tichelli *et al* (2002) evaluated outcomes among 36 pregnancies in women previously treated with immunosuppression for AA. They reported almost half involved a complication in the mother (three abortions, two cases each of eclampsia and maternal deaths) and/or baby (five premature deaths). Relapse of AA occurred in 19% and a further 14% needed transfusion during delivery. Normal blood counts before conception did not guarantee freedom from relapse of AA during pregnancy.

Better supportive care in recent years, particularly in supply of blood products, has led to improvements in maternal and fetal outcome (Kwon *et al*, 2006). However, it is important to discuss with the patient and family the potentially serious risks to both the mother and baby (Deka *et al*, 2003). It is essential that the patient be monitored frequently throughout pregnancy, initially monthly but later more frequently and according to disease severity, and with very close liaison with the obstetric team and haematologist. Presence of a PNH clone should warrant discussion with a specialist centre. The mode of delivery should be determined on obstetric grounds.

Supportive care is the mainstay of treatment of AA in pregnancy and the platelet count should, if possible, be maintained above 20×10^9/l with platelet transfusions. The high risk of alloimmunization and platelet refractoriness needs to be considered. CSA is safe during pregnancy (McKay & Josephson, 2006) and is recommended for those needing transfusions. ATG, allogeneic HSCT or androgens for AA during pregnancy are not recommended.

Key recommendations for management of AA in pregnancy

- **Supportive care remains the mainstay of treatment of AA in pregnancy, aiming to maintain the platelet count above 20×10^9/l with platelet transfusions. Grade 1C**
- **CSA is safe in pregnancy if needed. Grade 2C**

Paroxysmal nocturnal haemoglobinuria and AA

Tests to detect a PNH clone

Paroxysmal nocturnal haemoglobinuria should be excluded by performing flow cytometry (Parker *et al*, 2005; Borowitz *et al*, 2010). Analysis of GPI-anchored proteins is a sensitive and quantitative test for PNH, enabling the detection of small PNH clones which occur in up to 50% of AA patients, the proportion depending on the sensitivity of the flow cytometric analysis used (Dunn *et al*, 1999; Sugimori *et al*, 2006). Such small clones are most easily identified in the neutrophil and monocyte lineages in AA and will be detected by flow cytometry. If the patient has had a recent blood transfusion, a population of GPI-deficient red cells may still be detected by flow cytometry in the granulocyte and monocyte population. However, the clinical significance of a small PNH clone in AA as detected by flow cytometry remains uncertain. Such clones can remain stable, diminish in size, disappear or increase, hence the need for monitoring the clone. What is clinically important is the presence of a significant PNH clone often associated with clinical or laboratory evidence of haemolysis. Urine should be examined for haemosiderin as this is a constant feature of haemolytic PNH even when the patient does not have macroscopic haemoglobinuria. Evidence of haemolysis associated with PNH should be quantitated with the reticulocyte count, serum bilirubin, serum haptoglobin and lactate dehydrogenase. Patients should be screened for PNH at the diagnosis of AA. If persistently negative, test 6 monthly for 2 years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes, positive, test 3-monthly for the first 2 years and only reduce the frequency if the proportion of the PNH cells has remained stable.

The presence of a PNH clone in the setting of AA does not directly influence the choice of therapy for the underlying BMF. There is some evidence that the finding of a PNH clone predicts a better response to IST but this is not universal in all published reports. Patients with a significant PNH clone receiving IST, especially ATG, should be actively monitored for signs of haemolysis. Conversely, AA may later emerge in PNH patients in the presence of significant haemolysis.

New PNH patients should be referred to one of the two specialized nationally commissioned PNH centres, St James's University Hospital, Leeds, and King's College Hospital, London, to be assessed for PNH complications and for consideration for anti-complement therapy, following formal PNH National Service MDT review. Patients will be seen in either of the two national centres or in one of 10 Outreach clinics.

Data from the French Registry compared to the EBMT outcomes demonstrates that allogeneic stem cell transplant has an inferior outcome in haemolytic and thrombotic PNH compared to best supportive care including eculizumab when indicated (Peffault de Latour *et al*, 2012). Therefore the finding of a PNH clone does not affect positively or negatively on the decision to transplant.

Key recommendations for PNH and AA

- **All patients should be screened for PNH using flow cytometry on peripheral blood to detect deficiency of GPI anchored proteins, such as CD14, CD16, CD24 as well as FLAER for white blood cells, and CD55 and CD59 for red cell analysis.**
- **Patients should be screened for PNH at the diagnosis of AA. If persistently negative, test 6 monthly for 2 years and then move to annual testing unless symptoms/signs develop. If the PNH screen is, or becomes, positive, test 3-monthly for the first 2 years and only reduce the frequency if the proportion of the PNH cells has remained stable. Grade 2C**
- **Small PNH clones can be detected in up to 50% of patients with AA, usually without evidence of haemolysis; large clones are clinically significant and may result in haemolysis as well as increased thrombotic risk ('haemolytic PNH').**
- **Presence of a small/moderate PNH clone in AA does not directly influence the choice of treatment for the underlying BMF.**
- **New PNH patients should be referred to the PNH National Service to be monitored for PNH complications and assessed for anti-complement therapy.**

Disclaimer

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Committee for Standards in Haematology (BCSH) nor the publishers accept any legal responsibility for the content of these guidelines. These guidelines are only applicable to adult patients with AA.

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Author contributions

SBK chaired the guidelines group. JCWM was the senior author. All authors were involved in formulation, writing and approval of the guidelines. All authors approved the final version of the manuscript.

Conflict of interest

All authors have made a full declaration of interests to the BCSH and Task Force Chairs, which may be reviewed on request. In summary the following authors have declared the following conflicts of interest: SBK has received payment from Celgene for speaking at education meetings and from Novartis for speaking at education meetings and advisory work; TF has received payment from Alexion; ID has received payment from Life Length for lecturing; AK has received funding from Alexion for speaking at educational meetings; JM has received funding from Pfizer, Sanofi, Novartis and Alexion; GM has received funding from Celgene; JS has received funding from Merck Sharp and Dohme, Celgene, Orthobiotec and Pfzier. PH, AH have received payment from Alexion for lecturing. The rest of the authors have no declarations of interest.

Review process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BCSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BCSH guidelines website at www.bcshguidelines.com. If minor changes are required due to changes in level of evidence or significant additional evidence supporting current recommendations a new version of the current guidance will be issued on the BCSH website.

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·名家谈诊疗·

我如何规范诊治重型再生障碍性贫血

邵宗鸿

How I standardly diagnose and treat severe aplastic anemia *Shao Zonghong*

Corresponding author: Shao Zonghong, Department of Hematology, General Hospital, Tianjin Medical University, Tianjin 300052, China. Email: shaozonghong@sina.com

再生障碍性贫血(AA)是骨髓造血衰竭综合征的代表性疾病,细胞毒性T淋巴细胞功能亢进及其分泌造血抑制因子导致造血干细胞损伤是其发病的主要机制,其导致的造血微环境的改变加剧了疾病的发生和发展。AA的临床表现主要为因外周血细胞减少而导致的各种症状,如贫血、感染和出血。近年来,随着免疫抑制治疗(IST)和造血干细胞移植(HSCT)的进展,AA的治愈率和生存时间明显提高[1-3]。本文我们将通过1例年轻男性AA患者的诊疗过程,阐述笔者对如何规范诊疗AA的认识及经验。

患者,男,20岁,主因"面色苍白、乏力1个月"入院。既往无肝炎病史,无放射物质接触史,无长期服药史。入院查体:体温正常,贫血貌,四肢皮肤可见散在新鲜出血点,全身浅表淋巴结未触及肿大,肝脾肋缘下未触及。血常规:WBC 1.76×10^9/L,ANC 0.25×10^9/L,HGB 60 g/L,PLT 11×10^9/L,网织红细胞比值(Ret)0.27%。肝肾功能均无异常,血清铁、血清铁蛋白、叶酸和维生素B_{12}水平均正常。患者该如何诊治?

一、诊断及鉴别诊断

该患者血常规提示全血细胞减少,全血细胞减少的疾病主要包括:

1. 先天性骨髓衰竭综合征(CBMFS):患者多年轻,伴有先天性发育异常,如肢体畸形等。但不典型或者轻症者可以成人起病,没有明显畸形。我们对年龄<30岁骨髓衰竭患者常规进行染色体断裂试验。对疑似CBMFS的患者进行相关基因检测。

2. 骨髓增生异常综合征(MDS):患者年龄大、外周血和骨髓细胞发育异常,大部分骨髓增生活跃,原始细胞增多,部分有染色体异常。我们对所有骨髓衰竭患者进行骨髓和外周血涂片形态学检查、染色体核型分析(选择性MDS-FISH)、骨髓病理、MDS免疫表型分析和MDS常见基因突变检测。

3. 免疫相关性血细胞减少(IRP):患者骨髓细胞膜抗体阳性,外周血B淋巴细胞比例异常。部分患者可以检测到低滴度自身抗体(但不能诊断自身免疫性疾病)。我们常规进行骨髓有核红细胞、中性粒细胞和$CD34^+$细胞膜抗体检测进行鉴别。

4. 阵发性睡眠性血红蛋白尿症(PNH):典型患者伴有溶血发生,易鉴别。不典型患者我们采用流式细胞术检测外周血红细胞和白细胞CD59以及白细胞嗜水气单胞菌溶素变异体(Flaer)。

5. 低增生白血病、大颗粒淋巴细胞白血病、淋巴瘤、骨髓纤维化也可以出现全血细胞减少,若有特征性表现,不难鉴别。

6. 非血液系统疾病,如肿瘤骨髓转移和感染性疾病等也可以出现全血细胞减少,根据病史及相关实验室检查应易鉴别之。

本例患者外周血T淋巴细胞亚群比例为0.90,$CD4^+/CD8^+$比值为0.5。CD59及Flaer检测均阴性。丝裂霉素试验阴性。胸骨、髂骨骨髓涂片均为增生重度减低,正常形态淋巴细胞为主,无明显病态。骨髓活检:骨髓增生重度减低,无骨髓纤维化和异常细胞浸润。染色体核型:46XY[11]。骨髓细胞膜抗体阴性。病毒性肝炎检测均阴性。风湿抗体阴性,抗核抗体阴性。诊断:AA。

二、疾病严重程度评估

AA分为重型AA(SAA)和非重型AA(NSAA)。SAA诊断标准:①骨髓增生程度<正常的25%;②血常规需要具备下列3项中的2项:ANC<0.5×10^9/L,网织红细胞绝对值<20×10^9/L,PLT<20×10^9/L。ANC<0.2×10^9/L为极重型AA(VSAA)[4]。未达到SAA的AA为NSAA。根据骨髓和外周血指标,本例患者诊断为SAA。根据病史和相关检查,

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作者单位:300052 天津医科大学总医院血液内科
通信作者:邵宗鸿,Email:shaozonghong@sina.com

无明显并发疾病。

三、治疗选择

1. HSCT还是IST?

SAA一线治疗方法包括HLA相合同胞供者异基因HSCT(allo-HSCT)和IST。年龄<35岁、有HLA相合同胞供者的SAA患者可选择allo-HSCT作为一线治疗。年龄>50岁或不适合allo-HSCT的年轻SAA患者首选IST。年龄介于35~50岁的SAA患者可以根据个人意愿、治疗中心的技术水平选择HLA相合同胞供者allo-HSCT或者IST。IST失败后,有经验移植中心也可尝试进行替代供者移植。

本例患者为独生子,所以我们首选IST。

2. 用哪种免疫抑制剂?

目前治疗SAA的免疫抑制剂有抗胸腺细胞球蛋白(ATG)、抗淋巴细胞球蛋白(ALG)和环孢素A(CsA)。ATG/ALG根据种属来源分为马源、猪源和兔源。根据美国国立卫生研究院(NIH)和欧洲骨髓移植协作组(EBMT)的临床试验数据,马源ATG治疗初治SAA的疗效明显好于兔源ATG,因此美国和英国都将马源ATG作为优选一线治疗。NIH的Scheinberg等[5]比较了兔源ATG与马源ATG的疗效,6个月有效率:马源ATG 68%,兔源ATG 37%,差异有统计学意义(P<0.001)。3年总生存率:如果不进行HSCT,马源ATG 96%,兔源ATG 76%,差异有统计学意义(P=0.004);如果进行HSCT,马源ATG 94%,兔源ATG 70%,差异有统计学意义(P=0.008)。两组3年复发率与3年克隆演化率差异均无统计学意义。EBMT SAA工作组进行了一项多中心非随机非盲前瞻性的Ⅱ期临床试验,评估35例AA患者一线接受兔源ATG联合CsA治疗的疗效,以105例年龄与疾病严重程度相匹配的马源ATG联合CsA一线治疗的AA患者为历史对照进行比较:3个月时,兔源ATG组患者无一例获得完全缓解(CR),11例(34%)获得部分缓解(PR);6个月时,CR率为3%,PR率为37%。兔源ATG组10例患者死亡,其中1例出现在HSCT后,9例死于感染。最佳有效率:兔源ATG组为60%,马源ATG组为67%。2年总生存率:兔源ATG组为68%,马源ATG组为86%(P=0.009)。无移植生存率:兔源ATG组为52%,马源ATG组为76%(P=0.002)。多因素分析显示应用兔源ATG(HR=3.9,P=0.003)是生存的独立风险因子[6]。巴西进行的一项71例AA患者的回顾性研究中,接受马源ATG治疗的患者6个月有效率(60%)高于接受兔源ATG治疗的患者(36%),且前者有更高的生存率。在多因素分析中,兔源ATG是死亡的独立预测因子。此外,最近欧洲和美国进行的研究多数显示了兔源ATG治疗的AA有效率和生存率低[1]。

但也有部分临床试验数据显示兔源ALG疗效不劣于马源ATG。Manuel等20例兔ATG治疗的SAA患者与67例临床特征匹配的马源ATG治疗的SAA患者做对照,显示兔源ATG治疗组与马源ATG治疗组3、6、12个月的有效率是相似的,分别为40%与55%(P=0.43),45%与58%(P=0.44),50%与58%(P=0.61)。早期死亡率与总生存率差异无统计学意义。欧洲的一项研究也显示,马ATG作为一线治疗总有效率(49%)与兔ATG作为一线治疗总有效率(45%)差异无统计学意义。但此研究中马ATG有效率(49%)显著低于美国、欧洲、日本的前瞻性研究中的有效率(60%~70%)。亚洲的两个回顾性研究均显示兔源ATG与马源ATG有相似的有效率。国内有研究者分析了兔源ATG联合CsA治疗儿童AA的疗效,总共124例患者,总反应率是55.6%,反应中位时间为6个月,中位随访29个月,复发率和克隆演化率分别为3.2%及0.8%,5年总生存率为74.7%[1]。

无论马源ATG疗效是否优于兔源,我国目前暂无马源ATG可用。猪源ALG是我国研发的具有自主知识产权的产品,国内已有多个临床试验证实其治疗SAA的疗效至少不劣于兔源ALG[7]。

根据我国ATG/ALG供应情况和临床数据经验,我们选用国产猪源ALG治疗。具体剂量为25 mg·kg^{-1}·d^{-1},连用5 d。并联合CsA治疗,CsA开始时间为ALG治疗后第15天,初始剂量为3 mg·kg^{-1}·d^{-1},分次口服。根据血药浓度(100~200 μg/L)调整剂量。定期监测肝肾功能。

3. 造血刺激因子是否应用?

SAA大多存在粒细胞缺乏(粒缺)或重度粒缺,而粒缺的程度和时间是重度感染和死亡的最主要原因之一,因此迅速纠正粒缺状态是SAA治疗成功与否的关键因素之一。重组人粒细胞集落刺激因子(G-CSF)是快速升高粒细胞数量的细胞因子,可以减轻大部分SAA患者粒缺的程度,缩短粒缺的时间,减低感染率,缩短住院日期[1]。因此,对于SAA患者,在HSCT或IST基础上,应尽早应用G-CSF,升高ANC。应用G-CSF后3个月,如果粒缺未纠正,重做骨髓和染色体等克隆指标检查,核对SAA诊断是否可靠。若仍能排除MDS等非AA性疾病,

且骨髓三系无任何反应，可考虑第二次ATG治疗，但应选择另外种属ATG。如粒缺纠正，但其他两系无反应，可以将观察期延长至6个月。

该患者处于粒缺状态，我们给予G-CSF 300 μg/d，1周后，粒系有反应。2周后，ANC恢复至正常范围。调整G-CSF剂量，维持ANC在正常水平。

重组人血小板生成素（TPO）和TPO受体激动剂能促进巨核细胞增殖、分化和成熟，产生血小板。另外，造血干细胞上也存在TPO受体，因此TPO和TPO受体激动剂除了能升高血小板外，对于粒细胞和红细胞也有促进作用。国内IST联合TPO治疗初治SAA的临床试验显示，联合TPO治疗能促进SAA的恢复，提高有效率[8]。国外TPO受体激动剂治疗难治SAA和初治SAA的临床试验显示，其对于血小板、中性粒细胞和红细胞都有促进作用，明显提高IST的有效率[9-10]。国内也有报道IL-11对AA造血恢复有益处[11]。

本例患者治疗时，TPO受体激动剂艾曲波帕还没有上市。我们选用国产重组人TPO治疗。ALG治疗后第15天开始给药，15 000 U/d持续应用。

4. 雄激素是否应用？

雄激素能促进肾脏分泌EPO，增加有核红细胞对EPO的敏感性，促进红细胞增生刺激造血。在我国，雄激素仍然作为许多NSAA患者的一线治疗药物。目前常用的雄激素包括司坦唑醇、十一酸睾酮和达那唑。非对照的前期研究显示这些药物对部分患者有效，产生了持续的血液学恢复。对于IST无效和缺乏HSCT供者的患者，可以用雄激素治疗。近期研究显示，雄激素通过雌二醇芳香化为类固醇，作用于外周血淋巴细胞和$CD34^+$细胞TERT基因，增加端粒酶的活性，因此其在端粒缩短的AA患者中特别有用[12]。

本例患者应用十一酸睾酮治疗，40 mg，每日3次。持续应用。

四、疗效评价

根据我们的经验，SAA患者IST后，有效者多在3个月内ANC恢复至正常水平（G-CSF支持下），3个月后网织红细胞水平上升，血小板上升的时间变异性较大。绝大部分有效患者会在6个月内观察到疗效，少部分6个月后起效。根据目前通用疗效标准，血常规完全恢复正常水平并维持1年为基本治愈。脱离输血和不满足SAA标准为有效。

该患者IST后1个月ANC恢复正常（G-CSF支持下），IST后2个月网织红细胞比值恢复正常，IST后3个月网织红细胞绝对值恢复正常，IST后7个月PLT恢复正常。血常规正常后，缓慢停用CsA。目前患者血常规正常3年，未复发，基本治愈。

总之，SAA是一种致命的骨髓造血衰竭综合征，及时、精确诊断，选择合适的治疗可以显著改善预后。因为SAA发病率低，循证医学证据缺乏，建议患者去有丰富经验医学中心进行诊治。

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Research paper

Comparative study of porcine anti-human lymphocyte immunoglobulin and rabbit anti-human thymocyte immunoglobulin as a first-line treatment of acquired severe aplastic anemia

Miao Chen[a], Chao Liu[b], Xinhui Qiao[b], Daobin Zhou[a], Junling Zhuang[a], Bing Han[a,*]

[a] Department of Hematology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing, 100730, PR China
[b] LMIB of the Ministry of Education, School of Mathematics and Systems Science, Beihang University, Beijing, 100191, PR China

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ABSTRACT

Porcine anti-human lymphocyte immunoglobulin (pALG) and rabbit anti-human thymocyte immunoglobulin (rATG) are the only two ATGs for severe aplastic anemia (SAA) treatment in China. 148 treatment-naïve SAA patients who received ATG combined with cyclosporine A (CsA) therapy were analysed retrospectively. The patients were divided into a pALG group (n = 114) and a rATG group (n = 34). After three months, the pALG and rATG groups had an overall response (OR) of 65.8% and 44.1%, respectively (P = 0.023); after six months, the OR reached 74.6% and 64.7%, respectively (P = 0.361). The pALG group had markedly better time-related efficacy than the rATG group (P = 0.03). The overall survival (OS) and event-free survival (EFS) between groups had no significant difference (P > 0.1). The pALG and rATG groups did not significantly differ in terms of recurrence (8.8% vs. 5.9%, P = 0.734) or PNH clonal transformation (5.3% vs. 2.9%, P = 1.000), whereas a significant difference was found in the incidence of MDS/AML transformation (2.6% vs. 11.8%, P = 0.049). We found that pALG achieved a better time-related efficacy than rATG for the treatment of SAA; nonetheless, no significant difference in the OS or EFS of pALG compared with rATG.

1. Introduction

Acquired aplastic anemia (AA) is a bone marrow failure disease mainly due to T lymphocyte-mediated immune destruction of hematopoietic stem cells (HSCs), and it primarily manifests as pancytopenia and infection [1]. Immunosuppressive therapy (IST) is the first-line treatment of patients who are unsuitable for allogeneic HSC transplantation. The combined use of anti-human thymocyte immunoglobulin (ATG) and cyclosporine A (CsA) is now recognized as the standard intensive IST [2], which markedly improves the prognosis of patients with severe AA (SAA).

ATG is obtained by immunizing animals with cells from foetal or neonatal thymic tissues. Owing to the different species of immunized animals, ATG currently encompasses horse ATG (hATG), rabbit ATG (rATG) and porcine ATG (pALG), among various other types. The combination of hATG with CsA as a first-line treatment of SAA achieves a hematological response of 60–75% [3], while rATG as a second-line treatment achieves an overall response (OR) of 30–77% in patients who are non-responsive to hATG or who have recurrence after hATG therapy [4,5]. Several comparative studies, including prospective studies of hATG and rATG as first-line treatments for SAA showed that the therapeutic efficacy and survival are worse for rATG than for hATG [6–8], although rATG is generally thought to have a stronger immunosuppressive effect than hATG.

Currently, hATG is unavailable in the Chinese market. Since the 1980s, pALG, a formulation made in China, has been used to treat Chinese patients with SAA. The State Food and Drug Administration of China approved pALG for marketing in 2004, and pALG has been increasingly used widely; the efficacy of pALG is reported to reach 74.5–83.3% [9–11]. To date, only two retrospective comparative studies of rATG and pALG have been reported [12,13], and they suggested that the efficacy and survival of pALG are similar or superior to those of rATG when used as a first-line treatment of SAA. More abundant and sufficient data may change the suggested options for first-line drugs for intensive IST treatment of SAA in China.

Here, we report a retrospective study of pALG and rATG for the treatment of SAA. Ordinal logistic regression and COX regression analyses were conducted to compare the efficacy and survival between two groups of patients. Propensity score weighting was used to eliminate the non-randomness of the retrospective samples and control the potential influence of confounding variables on the efficacy assessment model. This study will provide more sufficient information for the

* Corresponding author.
E-mail address: hanbing_li@sina.com (B. Han).

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option of first-line treatment regimen for SAA patients in China.

2. Subjects and methods

2.1. Ethics statement

All procedures followed were in accordance with the ethical standards of the Ethics Committee of Peking Union Medical College Hospital and with the Helsinki Declaration of 1975, as revised in 2008. Patient information was anonymized and de-identified prior to analysis.

2.2. Subjects

A retrospective analysis was conducted on 148 treatment-naïve SAA patients (age > 12 years old) who were hospitalized at Peking Union Medical College Hospital and treated using pALG/rATG combined with CsA therapy from 1996 to 2015. The patients included 114 individuals who received pALG combined with CsA therapy (pALG group) and 34 who received rATG combined with CsA therapy (rATG group). AA diagnosis and severity determination were performed based on the diagnostic criteria defined by Camitta et al. [14,15], with congenital AA being excluded. The paroxysmal nocturnal hemoglobinuria (PNH) clone was defined as a positive acid hemolysis test or Ham's test (before 2007) or as neutrophils with CD59-negative cells ≥ 5% (from 2007 to 2010) or FLEAR-negative cells ≥ 1% (after 2010) along with the development of flow cytometry in our laboratory. Hemolytic PNH was defined as the presence of a significant PNH clone associated with clinical or laboratory evidence of hemolysis [2]. The presence of various infections, fever without a clear infection focus, brain hemorrhage, hepatic dysfunction (> 2 times the upper normal limit) and renal dysfunction at one week before intensive IST treatment was defined as a comorbidity during intensive IST treatment.

2.3. Methods

All patients underwent intensive IST treatment (pALG/rATG plus CsA) from 1996 to 2015, with no IST or only CsA therapy before enrollment. Allogeneic HSC transplantation was not possible due to the donor shortage or economic reasons. The subjects' data were collected from January 2013 to June 2016 and were analysed form June 2016 to September 2016. The treatment regimen was as follows: (1) pALG (Wuhan Institute of Biological Products Co., Ltd., Wuhan, Hubei, China): 20–30 mg/kg/day, for five days; rATG (Genzyme Polyclonals S.A.S, Lyon, France): 3–5 mg/kg/day, for five days; methylprednisolone 1 mg/kg/day was administered with dose tapering over one month to prevent serum sickness; (2) CsA: 3–5 mg/kg/day, with the blood trough concentration being monitored and maintained at ~200 ng/ml; the dose was gradually decreased after response, and the treatment was continued for more than two years; (3) adjuvant therapy: hematopoietic growth factors, such as granulocyte colony-stimulating factor, blood component transfusion and anti-infection therapy; and (4) antifungal prophylaxis for particular patients as determined by the physician, with fluconazole, itraconazole or posaconazole. Patients were re-examined after three, six and twelve months of IST treatment. We assessed the patients' clinical manifestations and re-examined their whole blood cells and reticulocytes, hepatic and renal function, CsA concentration and CD55 and CD59 on peripheral red blood cells and neutrophils. Bone marrow smears and biopsies were re-examined according to clinical indications. Thereafter, the assessment was carried out every 3–6 months. Nonresponsive patients were treated with a second course of intensive IST, or with traditional Chinese medicine or blood transfusion as supportive care. Recurrent patients were treated with a second course of intensive IST or with CsA only. Patients with transplantation as a rescue therapy were excluded in both groups.

2.4. Efficacy criteria

Efficacy was assessed according to the standards published by Marsh J.C.W. in 2009 [16] : (1) Complete response (CR): absolute neutrophil count > 1.5×10^9/L, hemoglobin > 110 g/L, platelet > 100×10^9/L; (2) Partial response (PR): no longer meeting criteria for SAA and not dependent on red blood cell or platelet transfusions; (3) Non-response (NR): continuous transfusion dependence after treatment without complete blood count improvement; (4) Recurrence: peripheral blood cell count reductions to SAA levels after CR or PR, regardless of transfusion dependence. When assessing the efficacy, hematopoietic growth factors were withdrawn for more than two weeks, and routine blood test parameters were maintained at stable levels for up to four weeks.

We defined complete response (CR) and partial response (PR) as "response", and non- response (NR) and death as "nonresponse". The speed of response is also an important feature that reflects medicinal properties and efficacy. Therefore, to evaluate the effect of time on efficacy, we used "response or not" and "time to response" to comprehensively assess the efficacy of intensive IST treatment. Therefore, a new efficacy variable was defined and assigned to be 0, 1 or 2. Specifically, 0 indicates "nonresponse" at both three and six months; 1 indicates "nonresponse" at three months but "response" at six months; and 2 indicates "response" at both three and six months. Thus, the outcome "2" indicates not only that a patient had a response but also that his/her response was rapid.

2.5. Statistical analysis

Data were statistically analysed using two statistical software packages, SPSS 20.0 (IBM SPSS, Somers, NY, USA) and R 3.3.1 (R Development Core Team, 2008). Two constituent ratios were compared using a contingency table chi-squared test, and data cells with an expected count less than five were analysed by Fisher's exact test. Measurement data with a normal distribution and equal variances were compared using the independent samples *t*-test, and measurement data that failed to show a normal distribution were compared using the Wilcoxon rank sum test. The Kolmogorov-Smirnov (K-S) statistic was used to test whether there was a significant difference in the distribution. An ordinal logistic regression model was adopted to compare the efficacy between two treatment groups. Survival analysis was conducted using the weighted Kaplan-Meier method, and the survival was compared using the weighted log-rank test. COX regression analysis was conducted to examine the difference in the survival between the two treatment groups. We also applied propensity score weighting to the above models to eliminate the non-randomness of retrospective data. Overall survival (OS) means the time from entry to any cause of death. And the time of last follow-up of the lost patients was analysed as the time of death. Event free survival (EFS) is the time from entry to any events including death, loss to follow-up, recurrence and clonal transformation (myelodysplastic syndrome, leukemia and paroxysmal nocturnal hemoglobinuria). A difference was considered statistically significant at $P < 0.05$.

3. Results

3.1. Patient characteristics

Table 1 shows the clinical characteristics of the patients in the pALG group (n = 114) and the rATG group (n = 34). The median age of pALG and rATG group was 30(12–72) and 35.5(14–76) respectively. Compared with the rATG group, the pALG group had a significantly higher pre-treatment absolute neutrophil (NEUT) count. No significant difference was found in the remaining characteristics shown in Table 1.

Table 1
Baseline characteristics of 148 patients received intensive IST.

Characteristics	pALG group	rATG group	P value
Patients (n)	114	34	
Sex			0.609
Male—n(%)	66(57.9%)	22(64.7%)	
Female—n(%)	48(42.1%)	12(35.3%)	
Median age (range)(years)	30(12–72)	35.5(14–76)	0.353
≦40—n(%)	80(70.2%)	24(70.6%)	0.963
> 40—n(%)	34(29.8%)	10(29.4%)	
Severity of AA			
SAA—n(%)	77(67.5%)	17(50.0%)	0.097
VSAA—n(%)	37(32.5%)	17(50.0%)	
HAAA	6(5.3%)	4(11.8%)	0.172
Median duration from AA onset to therapy (range) (months)	1.5(0.3–152)	2(0.5–60)	0.362
With complications before intensive IST	56(49.1%)	21(61.8%)	0.270
Antifungal prophylaxis	53(46.5%)	10(29.4%)	0.116
CBC before intensive IST			
WBC($\times 10^9$/L)	1.91 ± 1.10	1.51 ± 0.82	0.074
NEUT($\times 10^9$/L)	0.46 ± 0.46	0.26 ± 0.24	0.046*
LYM($\times 10^9$/L)	1.35 ± 0.82	1.19 ± 0.67	0.359
HGB(g/L)	67.10 ± 18.24	63.12 ± 18.56	0.754
MCV(fl)	91.83 ± 8.18	90.80 ± 6.67	0.788
RET($\times 10^9$/L)	19.66 ± 17.95	14.35 ± 11.42	0.288
PLT($\times 10^9$/L)	12.43 ± 8.27	11.15 ± 9.16	0.302
With PNH clone—n(%)	18(15.8%)	5(14.7%)	0.878
With symptomatic PNH—n (%)	2(1.8%)	0	0.592
Iron load before intensive IST			
SI	211.51 ± 64.40	211.15 ± 66.75	0.982
TIBC	269.26 ± 79.11	257.32 ± 71.73	0.524
TS	81.47 ± 19.96	84.61 ± 19.28	0.515
SF	805.66 ± 997.21	1053.90 ± 571.94	0.243

pALG porcine anti-lymphocyte immunoglobulin, rATG rabbit anti-thymocyte immunoglobulin, SAA severe aplastic anemia, VSAA very severe aplastic anemia, HAAA hepatitis-associated aplastic anemia, IST immunosuppressive therapy, CBC complete blood count, WBC white blood cell, NEUT neutrophil, LYM lymphocyte, HGB hemoglobin, MCV mean corpuscular volume, RET reticulocyte, PLT platelet, PNH paroxysmal nocturnal hemoglobinuria, SI serum iron, TIBC total iron binding capacity, TS transferrin saturation, SF ferritin.
* $P < 0.05$.

3.2. Hematological response

After three months of intensive IST treatment, the pALG and rATG groups had an OR (including CR and PR) of 65.8% and 44.1%, respectively (P = 0.023); after six months of treatment, the OR was 74.6% and 64.7%, respectively (P = 0.361). The hematological response in the two groups of patients is shown in Table 2. The preliminary results showed that similar efficacy was achieved in the two groups after six months of treatment, while more patients in the pALG group had a response by three months.

The efficacy was tested in the two groups of patients after the data were re-encoded using a time-related efficacy variable (as defined in Subsection 2.4 *Efficacy criteria*). A significant difference was found in the treatment outcome between the two groups (P = 0.047). The pALG group had a better treatment outcome than the rATG group.

As Table 1 showed, there were differences in AA severity, white blood cell (WBC) count and absolute NEUT count between the rATG and pALG groups ($P < 0.1$). These variables could be associated with treatment outcome. To eliminate the non-randomness of retrospective samples, we used the propensity score method. Based on the three confounding variables, we calculated the propensity score of each patient to balance the influence of covariates on treatment allocation. K-S statistic showed that propensity score weighting balanced the distribution difference of confounding variables between the two groups (Table 3).

The remaining covariates were added as control variables (including gender, age, body weight, with or without comorbidities during treatment, whether antifungal prophylaxis was used and lymphocytes (LYM), hemoglobin (HGB), mean corpuscular volume (MCV), platelet count (PLT) and reticulocyte count (RET) in pre-treatment blood cell counts) into the ordinal logistic regression model. The results showed that in the propensity score-weighted model, the efficacy of six month had no significant difference in both groups (P = 0.083), but the regression coefficient of the treatment group for the time-related efficacy was −1.06 (P = 0.03). The pALG group had a markedly better time-related efficacy than the rATG group.

Table 2
Hematological responses.

Time	Responses	pALG group	rATG group	P value
3 months	CR	6(5.3%)	2(5.9%)	0.055
	PR	69(60.5%)	13(38.2%)	
	NR	27(23.7%)	16(47.1%)	
	Death	12(10.5%)	3(8.8%)	
	OR (PR + CR)	75(65.8%)	15(44.1%)	0.023*
	No effect (NR + Death)	39(34.2%)	19(55.9%)	
6 months	CR	29(25.4%)	8(23.5%)	0.751
	PR	56(49.1%)	14(41.2%)	
	NR	16(14.0%)	8(23.5%)	
	Death	13(11.4%)	4(11.8%)	
	OR(PR + CR)	85(74.6%)	22(64.7%)	0.361
	No effect (NR + Death)	29(25.4%)	12(35.3%)	
Responses with time**	0	29	12	0.047*
	1	10	7	
	2	75	15	
At the end of follow-up	Relapse	10(8.8%)	2(5.9%)	0.734
	PNH transformation	6(5.3%)	1(2.9%)	1.000
	MDS/AML transformation	3(2.6%)	4(11.8%)	0.049*
	Death	22(19.3%)	10(29.4%)	0.238
	Loss to follow-up	9(7.9%)	3(8.8%)	1.000

pALG porcine anti-lymphocyte immunoglobulin, rATG rabbit anti-thymocyte immunoglobulin, CR complete response, PR partial response, NR none response, OR overall response, PNH paroxysmal nocturnal hemoglobinuria, MDS myelodysplastic syndrome, AML acute myeloid leukemia.
* $P < 0.05$.
** A new efficacy variable was defined by "response or not" and "time to response": 0 indicates "nonresponse" at both three and six months; 1 indicates "nonresponse" at three months but "response" at six months; and 2 indicates "response" at both three and six months.

The median follow-up time for patients who survived for more than 6 months were 70.3 months (6.1–138.5) and 29.85 months (9.1–158.6) for the pALG and rATG group, respectively. The incidence of myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) transformation significantly differed between the two groups (2.6% vs. 11.8%, P = 0.049). No significant difference was found in the incidence of other events, such as recurrence, PNH clone transformation, mortality and loss to follow-up.

3.3. Complete blood count changes after intensive IST treatment

After treatment, the complete blood cell count after one, three, six and twelve months of intensive IST treatment showed that the WBC, NEUT, HGB, MCV and PLT levels did not significantly differ between the two groups ($P > 0.1$). Nonetheless, the minimum value of lymphocytes was markedly lower for the rATG group (0.04 ± 0.05) than for the pALG group (0.11 ± 0.11) after intensive IST treatment ($P < 0.05$). The LYM of the rATG group remained significantly lower after one, three and six months of treatment and returned to the level of the pALG group after twelve months of treatment (Fig. 1).

Table 3
Balance test of the confounding factors before and after weighted of propensity score.

Confounding factors	rATG group	Before propensity score				After propensity score			
		pALG group	KS test statistics	P value	Effect size	pALG group	KS test statistics	P value	Effect size
NEUT	0.258	0.46	0.236	0.001	−0.861	0.284	0.073	0.571	−0.111
WBC	1.512	1.906	0.215	0.024	−0.479	1.504	0.073	0.958	0.011
Severity of AA: SAA	0.5	0.675	0.175	0.065	−0.351	0.551	0.051	0.625	−0.103

pALG porcine anti-lymphocyte immunoglobulin, rATG rabbit anti-thymocyte immunoglobulin, NEUT neutrophil, WBC white blood cell, AA aplastic anemia, SAA severe aplastic anemia.

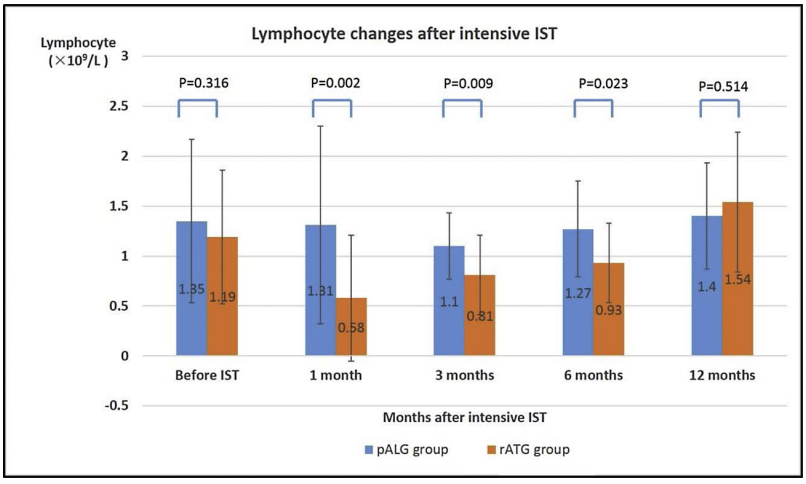

Fig. 1. Absolute lymphocyte changes of pALG group and rATG group in 1,3,6.12 months after intensive IST.

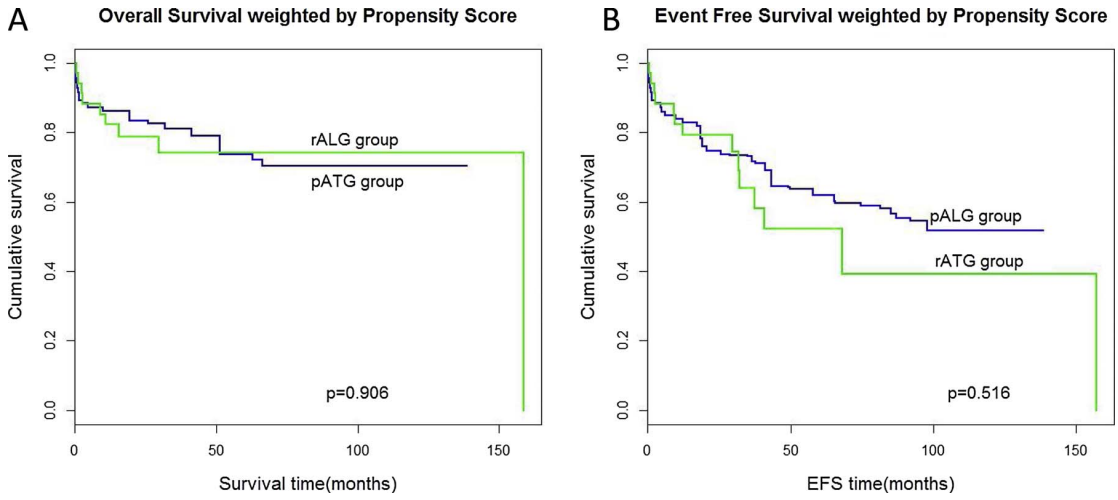

Fig. 2. A KAaplan-Meier survival curves of overall survival weighted by propensity score. B Kaplan-Meier survival curves of event free survival weighted by propensity score.

Table 4
Supportive treatment and side effects.

Characteristics	pALG group (N = 114)	rATG group (N = 34)	P value
Average length of stay (days)	33.58 ± 21.14	42.21 ± 18.94	0.036*
Incidence of infusion-related reactions	52.6%	67.6%	0.122
Incidence of serum sickness	14.0%	2.9%	0.122
Incidence of all infections in 3 months	52.6%	58.8%	0.525
Number of types of antibiotics	1.72 ± 2.03	2.30 ± 2.02	0.186
Number of days using antibiotics	12.00 ± 15.32	14.81 ± 14.99	0.391
Frequency of days using antibiotics	15.96 ± 23.20	20.74 ± 24.50	0.343
Number of types antifungal agent	0.34 ± 0.73	0.29 ± 0.54	0.730
Number of days using antifungal agents	4.62 ± 12.86	4.00 ± 9.76	0.815
Frequency of days using antifungal agents	5.67 ± 18.42	5.81 ± 15.76	0.971

3.4. Overall survival and event-free survival

The median follow-up time for patients who survived for more than 6 months was 51.4 months (6.1–158.6). The five-year survival was 80.5% (95% confidence interval (CI), 73.0–88.8%) for the pALG group and 74.1% (95% CI, 59.8–91.9%) for the rATG group. Propensity score-weighted overall survival (OS) and event-free survival (EFS) curves are shown in Fig. 2A and B, respectively. The two groups had no significant difference in OS (P = 0.906) and in EFS (P = 0.516). Propensity score-weighted COX regression with covariates showed that the regression coefficient of treatment group was 0.417 (P = 0.359) for OS and 0.447 for EFS (P = 0.315). That is, the two groups of patients did not significantly differ in OS and EFS.

Supportive care and treatment-related side effects

The supportive care and treatment-related side effects are compared between the pALG and rATG groups in Table 4. Drug infusion-related effects included fever, rash, facial oedema, vomiting, diarrhoea, suffocation, asthma, arrhythmia, hypotension and autoimmune hemolytic anemia, wherein fever included all types of febrile responses during infusion, without distinction between infusion-related fever and infection-induced fever. The infection included bacterial, fungal and viral infections. We compared the number of types and administration days of antibiotics and antifungal agents during therapy that were used from the beginning of intensive IST treatment until the discharge or death of the patients. Since two or more antibiotics or antifungal agents might be used simultaneously, we also compared the overall frequency of days using antibiotics and antifungal agents.

4. Discussion

Intensive IST treatment using ATG combined with CsA has significantly improved the prognosis of SAA patients who are not transplant candidates. Studies concerning the efficacy of hATG and rATG found that hATG is superior or equivalent to rATG [6,7]. The efficacy difference between hATG and rATG may be attributable to the differences in their immunosuppressive effects. rATG exerts a stronger immunosuppressive effect, leading to sustained suppression of lymphocytes [17], proliferation of regulatory T cells (Tregs) and increased expression of interleukin 10 [18]. However, hATG is still unavailable in the Chinese market, which only has rATG and pALG. pALG was chosen by many patients because of its relatively inexpensive price.

Thus far, only a few reports have retrospectively compared the efficacy of pALG and rATG. Ma et al. conducted a retrospective analysis on the efficacy and survival of seventy-seven treatment-naïve SAA patients who used pALG (n = 45) or rATG (n = 32) therapy [12]. The one-year OR was 83.78% for pALG-treated patients and 66.67% for rATG-treated patients (P = 0.036); the five-year OS was 82.22% for pALG-treated patients and 68.75% for rATG-treated patients (P = 0.32). Additionally, Liu et al. reported a retrospective analysis of the efficacy and survival of seventy-five treatment-naïve SAA patients who received pALG (n = 43) or rATG (n = 32) therapy [13]. The six-month OR was 66.67% and 61.29% for pALG-treated patients and rATG-treated patients, respectively (P > 0.05); the two-year OS was 87.4% and 83.2% for pALG-treated patients and rATG-treated patients, respectively (P > 0.05).

The present study analysed a greater number of cases for a comparative study of pALG and rATG for the treatment of SAA than analysed by previous studies. We developed a new variable that combined “response or not” and “time to response”; this time-related efficacy variable showed that the pALG group achieved a better treatment outcome than the rATG group. As a retrospective study, the two groups of patients showed differences in the distribution of disease severity, WBC count and absolute NEUT count. These differences may relate to treatment outcome [19]. Thus, we used propensity score-weighted statistical methods, which revealed the pALG group had a better time-related efficacy than the rATG group; nevertheless, no significant difference in the OS or EFS between groups.

In the current study, we found that the rATG group had a significantly lower minimum number of lymphocytes than the pALG group; the LYM of the rATG group remained significantly lower after one, three and six months of treatment and returned to the level of the pALG group after 12 months of treatment. These findings indicate more clearance of peripheral blood lymphocytes by rATG than by pALG, with a longer duration of persistence. However, similar to the comparative study of rATG and hATG, the stronger immunosuppressive effect did not improve the efficacy of treating SAA [7]. This result encourages further studies for the different effects of pALG and rATG on the immune system.

Although propensity score-weighted statistical analysis revealed that the pALG group received no significant benefit in terms of OS and EFS, a direct comparison of the follow-up results showed significant more malignant transformation of MDS/AML clones in the rATG group. The long-term complications of IST treatment include MDS/AML transformation, whose incidence was significantly higher than that in patients receiving hematopoietic stem cell transplantation (HST) (3.95% vs. 0.27%, P < 0.05) [20]. ATG therapy can increase Tregs and cause selective escape of cytotoxic T lymphocytes [17,18], which might lead to the appearance of abnormal clones. Studies on pALG have reported that the incidence of MDS/AML transformation is 0–2.9%, while the incidence of MDS/AML transformation after rATG therapy is 0–7.6% [9,11–13,21,22]. The previously reported difference may be mainly associated with the number of cases and the follow-up time. The risk of MDS transformation is increased in AA patients with specific disease characteristics, cytogenetic abnormalities or somatic mutations [23,24]. Unfortunately, we could not compare or balance this factor because molecular alterations were not assessed before treatment and the cytogenetic data were incomplete. Although the small number of cases in the rATG group and incomplete cytogenetic or somatic data should be considered when interpreting the results of our study, large-sample follow-up studies are worthwhile to illustrate whether there is a difference between pALG and rATG treatments in terms of the incidence of MDS/AML clone transformation.

Propensity score-weighted ordinal regression analysis was used to eliminate the non-randomness of the retrospective data. We found pALG achieved a significantly better efficacy than rATG for the treatment of SAA; nonetheless, no significant difference in the OS or EFS of pALG compared with rATG. This study provides information about the options for first-line drugs for intensive IST treatment of SAA patients in China who are not transplant candidates. The difference in the immunosuppressive effect of the two drugs on AA will be determined in further studies.

Conflict of interest

The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Long-term follow-up study of porcine anti-human thymocyte immunoglobulin therapy combined with cyclosporine for severe aplastic anemia

Miao Chen[1], Chao Liu[2], Junling Zhuang[1], Nong Zou[1], Ying Xu[1], Wei Zhang[1], Jian Li[1], Minghui Duan[1], Tienan Zhu[1], Huacong Cai[1], Xinxin Cao[1], Shujie Wang[1], Daobin Zhou[1], Bing Han[1]

[1]Department of Hematology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing; [2]LMIB of the Ministry of Education, School of Mathematics and Systems Science, Beihang University, Beijing, China

Abstract

Immunosuppressive therapy with antithymocyte immunoglobulin (ATG) and cyclosporine A is the first treatment option for severe aplastic anemia (SAA) patients without transplantation. Horse ATG is not marketed in China. Because the price of porcine ATG (pATG) is only about one-third of the price of rabbit ATG (rATG), long-term follow-up studies of pATG's efficacy will help provide valuable insights into the treatment of SAA. Retrospective studies were performed to analyze the clinical information of 102 SAA patients treated with pATG and cyclosporine A from 1999 to 2014 in Peking Union Medical College Hospital. The median age was 29 years old (range 12–72). Median follow-up time was 59.6 months (0.2–176.8). The overall response rate was 74.5% (CR 42.1%, PR 32.4%). The recurrence rate was 9.9%. The mortality rate was 16.7%. The median survival time has not been reached, and the 5-year survival rate was 81.8%. Other hematologic abnormalities were observed in 7.8% of patients, including symptomatic PNH, MDS, and AML. Multivariate analysis revealed there was no significant effect on survival by factors such as gender, age, severity of disease, treatment time, and PNH clone ($P > 0.05$). These data have indicated pATG therapy combined with cyclosporine A has significant long-term efficacy and high overall survival in SAA.

Key words severe aplastic anemia; immunosuppressive therapy; porcine anti-human thymocyte immunoglobulin; follow-up

Correspondence Bing Han, MD, Department of Hematology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing,100730, China. Tel: +861069155027; Fax: +861069155027; e-mail: hanbing_li@sina.com

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Acquired aplastic anemia (AA) is a syndrome of bone marrow hematopoietic failure characterized by pancytopenia and infection. Severe aplastic anemia (SAA) and very severe aplastic anemia (VSAA) patients suffer a high mortality rate (1). The incidence of acquired aplastic anemia in Europe and North America is approximately 2 per million per year, while the incidence is 2 to 3 times higher in East Asia (2). Young adult patients are becoming more common in China (3). It is believed that T-lymphocyte-mediated immunologic injury of hematopoietic stem cells is the main pathophysiologic cause of the disease (4). Bone marrow transplantation is the first option of therapy for SAA patients <40 years old with an HLA-matched sibling donor (5); however, due to economic reasons or lack of a matched donor, many patients, including young patients, are not candidates for bone marrow transplantation. Immunosuppressive therapy (IST) is becoming the first treatment option for those SAA patients without HLA-matched sibling donors. Combination treatment with antithymocyte globulin (horse or rabbit ATG) and cyclosporine A (CsA) is now considered as a standard intensive IST (2). Porcine ATG (pATG) has been used to treat patients with SAA in China since the 1980s. Porcine ATG is made using human fetal thymic cells as antigen, using swine as the animal for later immunity, separating antilymphocyte serum. The drug approval number is S10830001 by CFDA. The drug quality monitoring and control are strictly followed national law and have GMP certification. The research and production of porcine ATG began

in 1973 and clinical trials were carried out in several hospitals in China since 1978. Laboratory researches and clinical observations for more than 20 years show that the safety and efficiency of porcine ATG are similar as other ATGs. Because of its efficacy, safety, and relatively low cost, pATG is now widely used in China. In our preliminary study, the short-term effect of pATG plus CsA therapy for SAA was analyzed (6). In this study, we investigated the therapeutic effect of pATG plus CsA in SAA utilizing a larger study population and a longer follow-up time.

Patients and methods

Ethics statement

This study was approved by the Ethics Committee of Peking Union Medical College Hospital. Patient information was anonymized and de-identified prior to analysis.

Subjects

In this study, we analyzed 102 cases of SAA patients treated with pATG and CsA from 1999 to 2014 in Peking Union Medical College Hospital. The patients were given pATG as long as they were candidates and agreed with the regimen. Diagnostic criteria for AA followed those of Camitta (7). Patients who were younger than 40 years or who had a family history of bone marrow failure were carefully examined clinically and occasionally tested for chromosome breakage, telomere length, or telomerase gene mutations to exclude congenital AA. Patients who received stem cell transplantation as salvage therapy were excluded. Symptomatic paroxysmal nocturnal hemoglobinuria (PNH) was defined as a PNH clone of more than 1%, along with clinical and laboratory evidence of hemolysis (hemoglobinuria, increased reticulocytes, elevated plasma-free hemoglobin and lactic dehydrogenase, and hyperbilirubinemia). The PNH clone was detected by flow cytometry using anti-CD55 and anti-CD59 as described previously (8).

Research design

All patients received ATG treatment for the first time. Allogeneic hematopoietic stem cell transplantation was not available to them. All patients received pATG (Wuhan Biologics) 20–30 mg/kg/day for a total of 5 days via central venous catheter over a duration >8 h. Methylprednisolone 1 mg/kg/day was administered with dose tapering over 1 month to prevent serum sickness. Cyclosporine A was dosed at 3–5 mg/kg/day along with pATG, continuing at least 2 years, with serum drug concentrations maintained at approximately 200 ng/mL, followed by dose tapering. Supportive therapies including G-CSF, blood transfusions, and antibiotics were also used.

Efficacy criteria

All settings followed the 1998 Santa Margherita Ligure international standards to obtain clinical efficacy evaluations (9): (i) Complete response (CR): ANC>1.5 $\times$ 10^9/L, HGB>110 g/L, PLT>100 $\times$ 10^9/L; (ii) Partial response (PR): no longer meeting criteria for SAA and not dependent on red blood cell or platelet transfusions; (iii) Non-response (NR): continuous transfusion dependence after treatment without complete blood count improvement; and (iv) Recurrence: peripheral blood cell count reductions to SAA levels after CR or PR, regardless of transfusion dependence. Reaching CR and PR after treatment was considered evidence of efficacy. Responses were be confirmed when complete blood counts remained stable for 4 weeks after hematopoietic growth factors were discontinued for more than 2 weeks.

Follow-up

After IST treatments, patients were scheduled for follow-up visits every 3–6 months for 2 years. Afterward, there was an examination by clinic or telephone follow-up annually until either the patient's death or August 31, 2014. During each follow-up visit, patients were assessed by clinical examination, complete blood count with differential, reticulocyte count, hepatic and renal function, CsA concentration, CD55, and CD59. Bone marrow smears and biopsy specimens were re-examined as clinically indicated.

Statistical analysis

In this study, the data were analyzed by statistical software R 3.1.1. Count data were indicated by percentage (%). Chi-square analysis was used to measure and compare different factors influencing the efficacy. The Kaplan–Meier method was carried out to calculate the total survival rate by log-rank test to determine the significant effects of different factors on the survival time. Cox proportional hazards regression analysis was used to analyze the effects of factors on OS. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 102 patients received IST with pATG and CsA. Patient characteristics are shown in Table 1. Eight of 102 cases (7.8%) had PNH clones (CD55- and CD59-positive neutrophils ranged from 5–24%) at the onset of AA, and only 1 patient presented with symptomatic PNH. The median follow-up time was 59.6 months (range 0.2–176.8). Seven patients (6.9%) were lost to follow-up.

Table 1 Patients' characteristics

Characteristics	*N* (%)
Gender, male – no. (%)	62 (60.8%)
Median age – year (range)	29 (12–72)
≦40 – no. (%)	75 (73.5%)
>40 – no. (%)	27 (26.5%)
Etiology – no. (%)	
Idiopathic	98 (96.1%)
Hepatitis	4 (3.9%)
Disease Severity	
SAA	74 (72.5%)
VSAA	28 (27.5%)
Cell counts	
Absolute reticulocyte count/L	$18.3 \pm 2.5 \times 10^9$
Absolute lymphocyte count/L	$1.6 \pm 0.1 \times 10^9$
Absolute neutrophil count	
Mean/L	$0.3 \pm 0.09 \times 10^9$
$<0.2 \times 10^9$/L –no. (%)	28 (27.5%)
Platelet count/L	$14.2 \pm 2.2 \times 10^9$
PNH[1] clone – no (%)	
With PNH clone but no symptoms	7 (6.9%)
Symptomatic PNH	1 (1.0%)
Median months of follow-up(months)	59.6 (0.2–176.8)

Plus-minus values are means ± SD.
[1]PNH: paroxysmal nocturnal hemoglobinuria.

Hematologic response

Overall response (OR, including CR and PR) rate was 71.6% measured 3 months after intensive IST treatment and was 79.4% 6 months after intensive IST treatment. At the final follow-up (August 31, 2014), OR was 74.5%, as shown in Table 2.

Safety evaluation

Common side effects after pATG treatment were infusion-related hypersensitivity reactions and serum sickness (23 cases, 22.5%), characterized by fever, mild rash, or joint pain, and these side effects could be controlled by non-steroidal anti-inflammatory drugs. There were 2 cases (2%) of autoimmune hemolytic anemia induced by pATG, of which 1 case of hemolysis resolved after glucocorticoid treatment, and 1 patient died from secondary pulmonary infection after glucocorticoid therapy failed. Other major common complications included infections due to neutropenia, of which pneumonia was the most common. Thrombocytopenia led to skin and mucous membrane bleeding in some cases, while alveolar hemorrhage and hematuria occurred in some severe cases. A complete listing is provided in Table 3.

Table 2 Response rate of 102 SAA patients

	3 months No. (%)	6 months No. (%)	Final follow-up No. (%)
Response			
PR (%)	69 (67.6%)	58 (56.9%)	33 (32.4%)
CR (%)	4 (3.9%)	23 (22.5%)	43 (42.1%)
NR (%)	19 (18.6%)	10 (9.8%)	2 (1.9%)
OR[1](%)	73 (71.6%)	81 (79.4%)	76 (74.5%)
Relapse			8 (9.9%)
Death	10 (9.8%)	11 (10.8%)	17 (16.7%)
Lost to follow-up	0	0	7 (6.9%)

[1]OR=PR+CR.

Recurrence and clonal evolution

Treatments were effective in 81 patients (CR and PR) and the follow-up lasted for more than 6 months, including 8 recurrences (9.9%). Because there were 7 cases lost to follow-up, the calculated recurrence rate may be an underestimate. At the time of the final follow-up, median recurrence-free survival was not reached. Median follow-up time of relapsed patients was 65.8 months (36–96), and median time from pATG treatment to recurrence was 35.4 months (18.4–57.1). There were 6 patients with recurrence who received repeat treatment with cyclosporine and maintained PR until the final follow-up. There was one patient, lost to follow-up, for whom rabbit ATG treatment was unsuccessful. One patient died after recurrence.

There were 8 patients (7.8%) with other hematologic diseases. One patient (1%) was diagnosed with colon cancer at 57 months of follow-up. Five patients (4.9%) had symptom-

Table 3 Serious complications after ATG treatment and prognosis

Serious complications after treatment	Case (%)	Prognosis	
		Death(17)	Recovery
Infections	26 (25.5%)	13	13
Pulmonary infection	14	9	5
Bloodstream infection	5	4	1
Intracranial fungal infection	1		1
Oral soft tissue infection	1		1
Acute appendicitis	1		1
Perianal infection	2		2
Abscess gluteal region	1		1
CMV infection	1		1
Severe bleeding	3 (2.9%)	1	2
Cerebral hemorrhage	1	1	
Alveolar hemorrhage	1		1
Hematuria	1		1
Others	11 (10.8%)	3	8
Hepatotoxicity[1]	5		5
AIHA	2	1	1
Renal insufficiency	1		1
Ischemic heart disease	1	1	
Arrhythmia	1		1
Acute leukemia	1	1	

[1]ALT was more than 3 times higher than normal level.

atic PNH (CD59-positive cells from 0 to >20%, with hemolysis) over a median follow-up time of 28.8 months (24–40). They received glucocorticoid at acute attack of hemolysis and low-dose glucocorticoid maintenance. Two cases (1.9%) developed MDS-RA (normal karyotype) and MDS-RAEB (del 7) at 15 and 26 months, respectively. Erythropoietin had no effect on the former patient who lived on regular red blood cell transfusion. Chemotherapy as treating myeloid leukemia could not induce remission for the MDS-RAEB patient, and he died of infection. For one patient (0.9%), pATG therapy failed, and, 6 months later, the second course of IST (rATG) treatment also failed. Fifteen months after diagnosis, the patient developed acute myeloid leukemia (AML-M5), which had no response to DA regimen chemotherapy and died of infection.

Final survival

Seventeen patients died (16.7%), among whom median survival time was 2.3 months (0.2–36). Among patients who died, there were 6 cases of VSAA (including 1 case of post-hepatitis AA) and 11 cases of SAA. Ten patients died within 3 months, of whom 9 died from severe infections, and one died from AIHA. During the follow-up period, another 7 patients died due to infections, cerebral hemorrhage, ischemic heart disease, and progression to acute leukemia. The causes of death are shown in Table 3.

Median follow-up time for the 102 patients was 59.6 months (0.2–176.8), and seven patients were lost during the follow-up process, and the data of last time visit were used as a final status (4 PR and 3 NR cases). The overall 5-year survival rate after treatment was 81.8% (95% CI 74.2%, 90.2%). As of the final follow-up, median survival time has not been reached (Fig. 1). Five years of OS for VSAA patients was 78.6% (95% CI 64.8%–95.3%), while 5 years of OS for SAA patients was 83.9% (95% CI 75.5%, 93.2%) ($P > 0.05$). Median survival time was only 19.2 months for those patients experiencing serious complications within the first 3 months (not including pATG infusion-related hypersensitivity reactions and serum sickness, skin and mucous membrane bleeding, and febrile neutropenia easily controlled by broad-spectrum antibiotics). Median survival time in patients without complications has not been reached. Univariate analysis indicated that patient age ($P = 0.424$), gender ($P = 0.7239$), disease severity ($P = 0.2173$), time from onset to treatment ($P = 0.4387$), and presence of a PNH clone ($P = 0.7204$) had no significant effects on the survival time. Multifactor Cox risk modeling also revealed none of those factors had a significant effect on patient survival ($P > 0.05$).

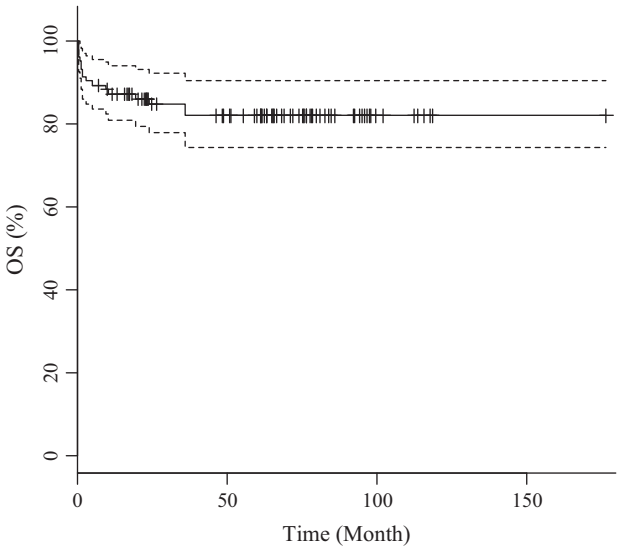

Figure 1 Kaplan–Meier Survival curves. Median survival time after treatment. Median follow-up time for this group of 102 patients was 59.6 months (0.2–176.8), and the overall 5-year survival rate after treatment was 81.8% (95% CI 74.2%, 90.2%). The dotted line represents the confidence interval.

Discussion

The present study includes the largest number of patients treated with pATG and the longest follow-up time published to date. The incidence of AA in China is higher than that in Europe and North America, but the economic situation of patients in China is far worse than that of patients in developed countries. Due to limitations of diagnosis and medical treatment in local regions, most of the SAA patients in China are treated at a small number of large research centers. Peking Union Medical College Hospital is one of the largest AA centers. For SAA patients who are not candidates for bone marrow transplantation, ATG plus CsA has become the consensus treatment. Currently, the most commonly used sources of ATG are horse and rabbit, and many retrospective studies have analyzed their efficacy. Six-month efficacy is 60–80% for horse ATG (10, 11) and 37–70% for rabbit ATG (12–14). The 5-year survival rate can be as high as 80–90% (15, 16). Recently, prospective studies have compared the effects of horse and rabbit ATG on AA and concluded that horse ATG is more effective than rabbit ATG (16–18). Therefore, there may be differences in the efficacy of ATG among different animal species.

Utilizing the large AA population in our hospital, for the first time, we analyzed pATG in combination with CsA for efficacy, survival, side effects, and risk factors influencing efficacy. During the median 59.6-month follow-up period, the overall response rate was 74.5%, slightly lower than the 83.3% we previously reported (6). The difference may be due to the larger sample and longer follow-up time of the current study. Compared with previously reported results for hATG and rATG, our data have indicated that pATG has at least comparable efficacy to ATG raised in other species (10–14). Our observations also demonstrated that pATG is

as safe as other types of ATG. Side effects such as fever, mild liver function abnormalities, serum sickness, and infection can be monitored and controlled (15). Prospective randomized controlled research deserves further investigation.

We showed that the recurrence rate was 9.9% and that the median time from recurrence to pATG treatment was 35.4 months (18.4–57.1). Our data showed low relapse rates compared to worldwide figures. In a prospective study, the cumulative recurrence rate at 3 years follow-up was 28% for horse ATG and 11% for rabbit ATG (no significant difference) (16). We notice that in other reports of porcine ATG, the relapse rates were also very low at 1.4–2.1% (6, 17). However, as 73.5% of the patients we analyzed were younger than 40 years old, we think that this may also contribute to the low relapse rate and good response rate. Of course, we also need longer follow-up time to show the final relapse rate. After most recurrences, cyclosporine was given again to maintain PR. As for clonal evolution, this study demonstrated that during the follow-up period, clonal evolution of blood diseases occurred in 7.8% of the patients, and one patient was diagnosed with colon cancer. In the absence of symptoms, we did not perform cytogenetic or genetic testing, potentially leading to an underestimate of MDS clonal evolution. However, those patients did not show myelodysplastic changes by bone marrow morphology. Reports in the literature indicate that clonal evolution after 3-year follow-up is 21% for hATG-treated patients and 14% for rATG-treated patients (19). There were no consistent mutations in patients with clonal evolution, and patients with monosomy 7 appeared to be relatively common (20), but some patients had that abnormality prior to treatment. Our data indicated that PNH evolution was found in 4.9% of patients; however, based on our study and other studies in the literature, PNH evolution can also be found in chronic AA patients receiving CsA treatment alone (21). There is no evidence that ATG treatment leads to PNH clone amplification (22). Given that the true incidence of clonal evolution after ATG reaches its peak at 10 years, our follow-up was too short to see the true incidence of clonal evolution.

Our survival analysis showed that the overall survival rate at 5 years after treatment was 81.8%. There was no significant difference in survival between VSAA and SAA patients. Severe complications within 3 months were critical factors for patient death, among which serious infection and bleeding were the most common. Aggressive supportive treatment, preventive antibiotic and antifungal therapy, and active platelet transfusions are important strategies to reduce early complications. In the analysis of the influencing factors on ultimate survival, we found that the time from diagnosis to treatment is not independent prognostic factors for survival. Because of its impact on the final efficacy, it could be important indicator for life quality of patients.

It is still controversial to define the difference of mechanisms of ATG from different species. *In vitro* studies of horse and rabbit ATG were previously conducted, and no difference was observed between species in terms of cytotoxity or antigen-binding specificity (23, 24). There was no difference between horse and rabbit ATG in the dynamic observation of depletion of lymphocytes *in vitro* tests (25). Recent *in vitro* studies showed that although both horse and rabbit ATG inhibited CD8 + cytotoxic T cells, rabbit ATG showed much stronger inhibition of CD4 + cells compared with horse ATG (16, 26). Certain cells in the CD4 + compartment may play an important role in hematopoietic recovery. Stronger inhibition of CD4 + cells by rabbit ATG may impede hematopoietic recovery. Interestingly, horse ATG is able to stimulate hematopoiesis while it inhibits immunity. However, few studies have compared pATG and rATG. One study suggests that pATG's drug metabolism curve has an advantage over rATG's curve in immunosuppressive therapy (27), but the underlying mechanisms are unknown.

In summary, our preliminary study and our present study have indicated that pATG, a less expensive form of immunosuppressive therapy, may be used as an alternative option for those SAA patients who are not bone marrow transplant candidates and that pATG's short-term efficacy, long-term efficacy, and safety are at least comparable to other ATG formulations. Prospective studies of larger samples, including head-to-head randomized control studies, are needed to compare different types of ATG for optimal treatment. These will eventually provide more evidence to further explore the underlying molecular mechanisms for ATG activities.

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ORIGINAL ARTICLE

Comparison of porcine anti-human lymphocyte globulin and rabbit anti-human thymocyte globulin in the treatment of severe aplastic anemia: a retrospective single-center study

Xiaorong Ma[1], Jin Wang[1], Wanggang Zhang[1], Xingmei Cao[1], Yinxia Chen[1], Aili He[1], Jie Liu[1], Nan Yang[2], Jianli Wang[1], Yun Yang[1], Yan Xu[1]

[1]Department of Hematology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shanxi Province; [2]The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shanxi Province, China

Abstract

Objectives: To compare the safety and efficacy of porcine antilymphocyte globulin (pALG) and rabbit antithymocyte globulin (rATG) in treating severe aplastic anemia (SAA). *Methods:* Seventy-seven patients with SAA that received immunosuppressive therapy between July 2004 and December 2013 at the Department of Hematology, the Second Affiliated Hospital of Xi'an Jiaotong University, were retrospectively analyzed. Forty-five patients received treatment including pALG (pALG group), and 32 patients received treatment including rATG (rATG group). Effective treatment rates between the two groups 1 yr after the treatment were compared; Kaplan–Meier 5-yr survival curve and log-rank test compared survival rates between the groups. All adverse responses were recorded. *Results:* The 1-yr overall response rate in the pALG group (83.78%) was significantly higher than that in the rATG group (66.67%, $P = 0.036$), and the 5-yr overall survival rate in the pALG group (82.22%) was also higher than that in the rATG group (68.75%), but the difference was not statistically significant ($P = 0.32$). The incidence of adverse events was similar in the two groups, and no treatment-related deaths occurred. *Conclusions:* The efficacies, survival, and safety profiles of pALG-based treatments are similar to or even better than those of rATG-based treatments. These results may help guide the clinical use of pALG in immunosuppressive therapy for SAA.

Key words immunosuppression; anemia; aplastic; treatment outcome

Correspondence Xiaorong Ma, Department of Hematology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, Shanxi Province, China. Tel: +13992856156; Fax: +86 21 64085875; e-mail: maxrsci@sina.com

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Aplastic anemia (AA) is an acquired bone marrow failure syndrome, characterized by decrease of all three blood cell types in the peripheral blood. AA is caused by abnormalities in the bone marrow hematopoietic stem cells/hematopoietic progenitor cells (1). Severe aplastic anemia (SAA) is usually fatal unless treated effectively, but current treatment strategies mean that up to 90% of patients can expect to live for years after diagnosis (2).

There are two preferred treatment methods for SAA: hematopoietic stem cell transplantation (HSCT) and immunosuppressive therapy (IST) (2). HSCT is the method of choice for younger patients with suitable donors. IST is the first-line treatment strategy for patients that lack human leukocyte antigen (HLA)-identical sibling donors (3, 4).

IST is usually based on antithymocyte globulin/antilymphocyte globulin (ATG/ALG) polyclonal antibodies from the serum of animals immunized by human thymocytes/lymphocytes. The standard IST regimen involves horse antithymocyte globulin (hATG) and cyclosporine (CSA) (5). A total of 60–75% of patients with SAA receiving first-line IST show hematological improvement 6 months after initial treatment (6–10), with 75–85% 5-yr survival (11). For patients that do not respond to initial treatment with hATG, salvage treatment with rabbit antithymocyte globulin (rATG) results in beneficial effects in about 30–77% of patients (12, 13).

For patients who initially responded but later relapsed, rATG treatment results in a secondary hematological response in 65% of patients (13, 14). These results show that IST benefits about two-thirds of the patients with SAA that cannot be treated by bone marrow transplantation.

The use of rATG may not be quite as effective as first-line treatment involving hATG (5). But in some countries, hATG has recently become unavailable, so rATG is the only option for first-line treatment (15). Alternative IST regimens have also been proposed in an effort to improve SAA treatment, and many different antibodies have emerged (5). One of these alternatives is porcine antilymphocyte globulin (pALG) that is widely used in China (16, 17), in part because it is less expensive than rATG.

The aim of this study was to compare the use of pALG and rATG in terms of the 1-yr efficacies, 5-yr survival, and safety profiles. We also investigated the clinical factors that affected the survival of the patients to provide evidence for the selection of the animal species in choosing ATG/ALG in clinical practice and to guide further studies on the mechanisms of ATG/ALG from different animal species.

Patients and methods

Patients

This was a single-center, non-randomized, retrospective cohort study designed and conducted by the Department of Hematology, the Second Affiliated Hospital of Xi'an Jiaotong University. Seventy-seven patients with SAA that received IST between July 2004 and December 2013 in the Department of Hematology, the Second Affiliated Hospital of Xi'an Jiaotong University, were included. The inclusion criteria were as follows: patients (i) diagnosed with SAA; (ii) with a lack of human leukocyte antigen (HLA)-histocompatible siblings as donors for bone marrow transplantation; (iii) who were ATG/ALG+CSA-based IST naïve; and (iv) aged ≥4 yr. The exclusion criteria were as follows: patients (i) diagnosed with Fanconi anemia, dyskeratosis congenital (DKC) bone marrow failure syndrome, or congenital bone marrow failure syndrome; (ii) with clinical evidence suggesting myelodysplastic syndrome (MDS); (iii) with clinical evidence suggesting paroxysmal nocturnal hemoglobinuria (PNH)-typical hemolysis, with a history of PNH-related thrombosis, or flow cytometer examination showing PNH clones >50%; (iv) who were pregnant or breastfeeding women; (v) with severe, uncontrollable infection or unexplainable fever (>38°C); and (vi) with a history of malignant tumors, or with severe complications (expected survival ≤3 months) of the liver, renal, heart, or metabolic systems.

Patients were diagnosed with AA if they met at least two of the following items: (i) peripheral hemoglobin concentration <100 g/L; (ii) peripheral platelet count $<50 \times 10^9$/L; (iii) peripheral neutrophils count $<1.5 \times 10^9$/L; and (iv) bone marrow biopsy showed hemopoietic tissue decreases and fat tissue increases. Patients with a bone marrow cell count <25%, or between 25% and 50% but the residual hematopoietic cells <30%, and met at least two of the following items were diagnosed as SAA: (i) absolute value of reticulocytes $<20 \times 10^9$/L; (ii) neutrophils $<0.5 \times 10^9$/L; and (iii) platelet $<20 \times 10^9$/L. Patients were diagnosed with very severe aplastic anemia (VSAA) if they met the diagnostic criteria of SAA and the neutrophils $<0.2 \times 10^9$/L (18).

This study was approved by the ethics committee and medical academic staff committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Informed consent was obtained from all of the patients or their legal guardians before participation in this study.

Study design

Grouping of the patients: All 77 patients were sufficiently informed of the two agents to be used and were allowed to choose pALG or rATG as the basis of their treatment. The patients were allocated into the pALG group and the rATG group according to the treatment strategy they had chosen.

Treatment strategies: For the pALG group, intravenous injection of pALG (Wuhan Institute of Biological Products Co. Ltd., Wuhan, China) at a dose of 25 mg/kg/d was performed for 5 continuous days, while for the rATG group, 2.5 mg/kg/d of rATG (Thymoglobulin; Genzyme Corp., Cambridge, MA, USA) was intravenously injected for 5 continuous days. Oral administration of antihistamine drugs was performed for all of the patients before the intravenous injection to prevent allergic reactions. Intravenous injection of prednisone (1 mg/kg/d) was also performed from day 1 to 14, and then, the dose was halved every 5 d until it was stopped on day 28–30 to prevent serum sickness. Oral administration of CSA (Novartis International AG, Basel, Switzerland) was performed from day 6 (2–3 times/d; 3–4 mg/kg/d for adults, and 5 mg/kg/d for children) to maintain a minimum blood CSA concentration between 150 and 250 ng/ml for 6–12 months, and then, the dose of CSA was decreased gradually after all the three blood cell types were stabilized; the overall treatment duration for the patients was at least 2 yr. Except for ATG or ALG, the other treatments for the patients including glucocorticoids, CSA, androgen, hematopoietic growth factors, blood transfusion, anti-inflammatory therapy, and supporting therapy were all identical. The detailed treatment strategies are shown in the Appendix S1 of the supplementary material.

Follow-up: The endpoint for the efficacies was the effectiveness of the treatment 1 yr after IST treatment; the endpoint for survival was patient mortality 5 yr after IST.

Evaluation criteria: The efficacies were evaluated according to the results of two or more routine blood examinations

that were performed within 4 wk; the patients should have stopped hematopoietic growth factor treatments for at least 1 wk when examinations were carried out. The evaluation criteria were defined as follows: Complete response was (CR) when all three blood cell types in the peripheral blood were restored to levels of people of the same age and sex: hemoglobin $\geq$ 120 g/L (male) or 110 g/L (female); neutrophil $\geq 1.5 \times 10^9$/L; and platelet $\geq 100 \times 10^9$/L. Partial response (PR) was when blood transfusion was not required and the patient no longer met SAA criteria. Overall response (OR) was the total number of CR and PR. No response (NR) was when blood transfusion was required, or the patient still met the SAA diagnostic criteria. Recurrence was when a patient achieved hematological remission with blood cell levels maintained for more than 3 months and then routine examinations showed that the blood cell levels decreased to meet the diagnostic criteria of SAA. PNH transformation showed positive results at least twice in Ham's tests, or two blood cell types showed more than 20% glycosylphosphatidylinositol-anchored protein (GPI-AP) deletion, with clinical or laboratory-confirmed hemolysis. MDS transformation was when at least two blood cell types in the bone marrow showed dyshematopoiesis, with or without abnormal chromosome karyotype. A PNH clone was when flow cytometer examination showed more than 5% GPI-AP deletion. Early death was when a patient died within 3 months of ATG/ALG treatment (6).

Sampling methods: To evaluate the efficacies of the treatment, as well as drug-related liver and renal function damage, blood examinations were performed for the patients every 3 d within 6 months after IST, then once every week at 6–12 months, and at least once every month after 1 yr. Liver and renal functions were examined every week within 6 months after IST, then once every 2 wk at 6–12 months, and at least once every month after 1 yr.

To evaluate the recovery of bone marrow hematopoietic functions and cellular immunity, reticulocytes and T-cell subsets were examined before and at 1 wk, 2 wk, 1 month, 3 months, and 6 months after IST.

To identify late complications or MDS, AML, and PNH transformation, bone marrow aspiration and biopsy, chromosome karyotype examination and GPI-AP deletion examination were performed at 3 months after IST. The examinations were repeated every 6 months if the results were stable; however, for cases with abnormal changes in blood routine examination, these examinations were repeated immediately.

Statistical analyses: SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Student's *t*-test or Mann–Whitney test was used for the comparisons of the quantitative data between the pALG and rATG groups; Pearson's chi-square test or continuity-corrected chi-square test was used for the comparisons of the rates between the two groups. Kaplan–Meier method was used to estimate the 5-yr survival, and log-rank test was used for the comparison between the two groups. Cox regression was used for the multivariate analysis of the prognoses of the patients. All the statistical analyses were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Characteristics of the patients

No significant difference in background characteristics, treatments before ATG/ALG, and the time interval between the diagnosis and treatment was found between the two groups ($P > 0.05$). The duration of CSA treatment and the CSA level at 6 months were also not significantly different between the two groups ($P > 0.05$) (Table 1). All 77 patients were followed up until the efficacy endpoint. Seventy-four patients were followed up until the survival endpoint, and three patients were lost to follow-up (3.89%). All of the three patients who were lost to follow-up were not responding to the treatment at the last follow-up. After the patients that died within 1 yr after the treatment were excluded, the median follow-up time was 1622 (387–1925) d and 945 (493–1741) d in the rATG and pALG groups, respectively, and the difference was not statistically significant ($P = 0.18$).

Efficacy evaluation

Mortality and the causes of death: Eight patients in the rATG group died within 5 yr of treatment, a mortality rate of 25%; 5 (15.63%) of those had an early death. Seven (15.56%, $P = 0.302$) patients in the pALG group died within 5 yr of treatment, among which 5 (11.11%, $P = 0.813$) patients had an early death. For the patients that died, the treatment was rated as non-effective. The major cause of death was infection, which was directly associated with 80% of the deaths. The mortality and the causes of deaths are listed in supplementary Appendix S2.

One-year efficacy and the restoration of hematopoiesis

The data from the remaining 27 patients in the rATG group were used for the evaluation of efficacy, which showed that the 1-yr overall response rate was 66.67%. While for the pALG group, seven patients died and one had experienced transformation, the data of the other 37 patients were used for the evaluation of efficacy, which showed that the 1-yr overall response rate was 83.78%. The 1-yr overall response rate was significantly higher in the pALG group than in the rATG group ($P = 0.036$). The efficacies at the other time points are shown in

Table 1 General characteristics of the 77 patients receiving IST

Characteristics	pALG group	rATG group	*P*-value[1]
n	45	32	
Median age (years)	21 (4~56)	24.5 (7~64)	0.46
Age (years)			
<40	37 (82.22%)	26 (81.25%)	0.91
≥40	8	6	
Sex			
Male	29 (64.44%)	19 (59.38%)	0.65
Female	16	13	
Disease classification			
VSAA	14 (31.11%)	13 (40.63%)	0.39
SAA	31	19	
Causes of the disease			
Idiopathic AA	43 (95.56%)	32 (100%)	0.63
Hepatitis-related AA	2	0	
Pretreatment hemogram			
ANC ($\times 10^9$/L)	0.24 (0.02~1.03)	0.37 (0~1.0)	0.98
Ret (%)	0.12 (0.03~1.73)	0.16 (0.07~1.52)	0.34
Ret ($\times 10^9$/L)	7.3 (0~18.9)	11.3 (0~19.8)	0.53
PLT ($\times 10^9$/L)	13 (2~20)	11.5 (6~20)	0.89
PNH clone	0	0[2]	
Chromosome abnormality	0	0[3]	
Treatments before IST			
Yes	4 (8.89%)	4 (12.5%)	0.90
Androgen ± CSA	3	4	
IVIg ± glucocorticoids	1	0	
Median time from diagnosis to treatment (days)	13 (3~376)	25 (7~1135)	0.10
<30	35 (77.78%)	20 (62.5%)	0.14
≥30	10	12	
Median duration of CSA treatment (days)	632 (153~981)	533 (106~1012)	0.54
Blood concentration of CSA at 6 months	178 ± 47.4	193 ± 72.3[4]	0.43
Follow-up time (patients died within 1 year after the treatment were excluded)	945 (483~1741)	1622 (387~1825)	0.18

pALG, porcine antilymphocyte globulin; rATG, rabbit antithymocyte globulin; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia; AA, aplastic anemia; ANC, absolute neutrophil count; Ret, reticulocyte; PLT, platelet; PNH, paroxysmal nocturnal hemoglobinuria; IST, immunosuppressive therapy; IVIg, intravenous immunoglobulins; CSA, cyclosporine.

[1]$P < 0.05$ indicates that the difference between the two groups is statistically significant.

[2]GPI-AP deletion was examined by flow cytometer for only the 27 patients that were treated after 2012, among which 15 were in the pALG group and 12 were in the rATG group.

[3]Chromosome abnormality examination was performed only for the 32 patients that were treated after 2012, among which 18 were in the pALG group and 14 were in the rATG group.

[4]Blood CSA concentration was examined for 28 and 20 patients in the pALG and rATG groups at 6 months after the treatment, respectively, while 13 and 9 patients in the pALG and rATG groups did not receive such examination because the CSA treatment was discontinued or the dose was reduced.

Table 2. The median time for achieving the first effectiveness was 94 (17–265) and 146 (31–334) d for the patients in the pALG and rATG groups, respectively, and the difference was statistically significant ($P = 0.03$).

The median time for achieving absolute neutrophil count >1.0 × 10^9/L, discontinuation of red blood cell transfusion, and discontinuation of platelet transfusion was 12 (0–27), 35 (0–131), and 82 (16–265) d for the patients in the pALG group, and was 16 (0–36), 56 (27–167), and 106 (29–334) d for the patients in the rATG group, respectively. Restoration of hematopoiesis was earlier in the pALG group than in the rATG group, but the difference was not statistically significant ($P = 0.87$, $P = 0.62$, and $P = 0.41$).

Recurrence

No cases of recurrence were found in the rATG group; however, 3 were found in the pALG group (6.67%, $P = 1.00$ compared to rATG group). The time of recurrence was day 147, 283, and 540, respectively. No secondary IST or bone marrow transplantation was performed for these three patients; instead, the duration of CSA treatment was

Table 2 Efficacies of the treatments

	Complete Recovery		Partial Recovery		Overall Recovery		No Recovery		Dead		Relapse		Transformation		Lost to follow-up	
	pALG (n = 45), (%)	rATG (n = 32), (%)	pALG (n = 45), (%)	rATG (n = 32), (%)	pALG (n = 45), (%)	rATG (n = 32), (%)	pALG (n = 45), (%)	rATG (n = 32), (%)	pALG (n = 45)	rATG (n = 32)	pALG (n = 45)	rATG (n = 32)	pALG (n = 45)	rATG (n = 32)	pALG (n = 45)	rATG (n = 32)
1 month	0	0	3 (7.14)	1 (3.45)	3 (7.14)[1]	1 (3.45)	39 (92.86)	28 (96.55)	3	3	0	0	0	0	0	0
3 months	0	3 (11.11)	22 (55.00)	6 (22.22)	22 (55.00)[2]	9 (33.33)	18 (45.00)	18 (66.67)	5	5	0	0	0	0	0	0
6 months	3 (7.89)	3 (11.11)	26 (68.42)	10 (37.02)	29 (76.32)[3]*	13 (48.15)	8 (21.05)	14 (51.85)	6	5	1	0	1	0	0	0
1 year	10 (27.03)	11 (40.74)	21 (56.76)	7 (25.93)	31 (83.78)[4]*	18 (66.67)	4 (10.81)	9 (33.33)	7	5	2	0	1	0	0	0

pALG, porcine antilymphocyte globulin; rATG, rabbit antithymocyte globulin.
$P < 0.05$ indicates that the difference is statistically significant.
[1]$P = 0.889$ indicates no significant difference between the pALG and rATG groups.
[2]$P = 0.081$ indicates no significant difference between the pALG and rATG groups.
[3]*$P = 0.012$ indicates significant difference between the pALG and rATG groups.
[4]*$P = 0.036$ indicates significant difference between the pALG and rATG groups.

increased, and the patients achieved PR again on day 262, 570, and 433, respectively; in addition, two of the three patients finally achieved CR.

Survival

The 5-yr overall survival rate was 82.22% and 68.75% ($P = 0.169$) and event-free survival rate was 71.11% and 68.75% ($P = 0.823$) for the patients in the pALG and rATG groups, respectively. Two patients in the pALG group and four patients in the rATG group had no response at the end of the follow-up; however, no secondary IST or bone marrow transplantation was performed; instead, blood transfusion was performed to keep them alive. The survival rates at the other time points are listed in supplementary Appendix S3, and the 5-yr survival curves are shown in Fig. 1.

Transformation

Two patients in the pALG group had undergone transformation at the end of the follow-up, while no transformation was found in the rATG group ($P = 0.63$). Reexamination of the bone marrow cells with fluorescent *in situ* hybridization (FISH) showed a 5q31 deletion in one patient, which was diagnosed as MDS on day 125. The other patient showed PNH-typical hemolysis and thrombosis, and flow cytometer examinations also showed a GPI-AP deletion >20%; thus, this patient was diagnosed with PNH on day 455. Neither of these patients achieved effectiveness before transformation, and blood transfusion was performed to keep them alive.

Multivariate analysis of prognosis

Multivariate analysis (Table 3) showed that age, sex, pretreatment hemogram, other treatments before IST, and the

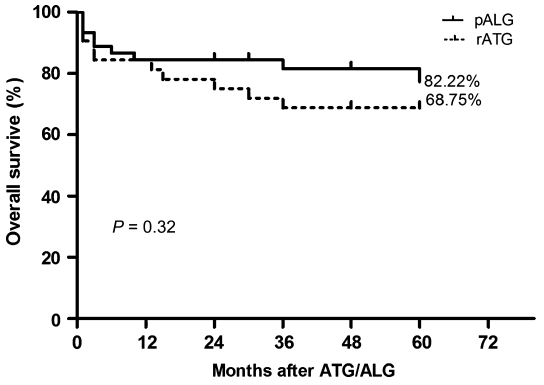

Figure 1 Kaplan–Meier survival analysis. Overall survival of the two groups of patients in the months after antithymocyte globulin/antilymphocyte globulin (ATG/ALG) treatment: pALG group treated with porcine antilymphocyte globulin, and rATG group treated with rabbit antithymocyte globulin. There was no significant difference between the groups ($P = 0.32$).

Table 3 Multivariate analysis of the factors affecting patient prognosis

Clinical characteristics	*P*-value	OR (95%CI)
Age ≥40 years	0.87	0.92 (0.27–2.87)
Male	0.97	1.03 (0.13–7.74)
VSAA	0.04*	2.50 (1.03–5.68)
Pretreatment hemogram		
ANC $<0.5 \times 10^9$/L	0.07	2.47 (0.91–6.68)
PLT $\leq 10 \times 10^9$/L	0.47	1.53 (0.47–4.92)
HB <60 g/L	0.35	1.77 (0.52–6.01)
Time from diagnosis to treatment ≥30 d	0.01*	3.60 (1.50–10.10)
Treatments before IST	0.34	1.49 (0.65–3.40)
pALG vs. rATG	0.32	0.63 (0.25–1.58)
Non-responder at 6 months	0.01*	3.75 (1.03–6.04)

OR, odds ratio; 95% CI, 95% confidence interval; VSAA, very severe aplastic anemia; ANC, absolute neutrophil count; PLT, platelet; HB, hemoglobin; pALG, porcine antilymphocyte globulin; IST, immunosuppressive therapy; rATG, rabbit antithymocyte globulin.
*$P < 0.05$ indicates that the difference is statistically significant.

Table 4 Adverse responses and toxicities

Adverse response	pALG group	rATG group	*P*-value*
Allergic reaction	5	3	1.00
Serum sickness	17	12	0.98
Fever	10	9	
Rash	3	1	
Arthralgia	4	2	
Infection	30	21	0.54
Bleeding	6	4	1.00
Liver function damage	14	9	0.78
Renal function damage	3	0	0.37
Hypertension	7	3	0.65
Impaired glucose tolerance or fasting glucose	2	1	1.00
Others	1	2	0.76

pALG, porcine antilymphocyte globulin; rATG, rabbit antithymocyte globulin.
*$P < 0.05$ indicates that the difference is statistically significant between the two groups.

species of ATG/ALG did not significantly affect patient prognosis, while VSAA, time from diagnosis to treatment ≥30 d, and non-responder at 6 months were independent factors that affected prognosis. The possibility of death within 5 yr was 1.5-fold higher in the patients with VSAA than in those with SAA ($P = 0.04$, 95% CI: 1.03–5.68), was 2.6-fold higher in the patients with time from diagnosis to treatment ≥30 d than in those with time <30 d ($P = 0.01$, 95% CI: 1.50–10.10), and was 2.75-fold higher in the non-responders at 6 months than in the responders ($P = 0.01$, 95% CI: 1.03–6.04).

Treatment-related adverse responses and toxicity evaluation

The incidence of adverse responses shown in Table 4 was not significantly different between the pALG and rATG groups ($P > 0.05$). All were mild and controllable, and recovered after symptomatic treatment. No treatment-related deaths occurred.

Mechanisms of the effects of ATG/ALG

The absolute lymphocyte count (ALC) is shown in Fig. 2 and was not significantly different between the pALG and rATG groups before the treatment; however, it was significantly different between the 2 groups at the day 1, day 3, day 5, day 7, wk 2, month 1, month 2, month 3, month 4, month 5, and month 6 after the treatment ($P < 0.05$). The difference in the percentage of T-cell subsets was not significant between the two groups before the treatment; however, the percentage of CD3+ T cells was significantly different between the two groups at 2 wk after the treatment, and the percentage of CD4+ T cells was significantly different between the 2 groups at 2 and 3 wk as well as 1 and 2 months after the treatment ($P < 0.05$).

Discussion

The aim of this study was to compare the treatment of SAA with two different treatment regimens. One regimen included pALG and the other rATG. The results show that in terms of efficacy, pALG had a significantly better overall response rate than rATG and showed faster median time to achieve first effectiveness. In terms of overall survival and safety, there were no significant differences between the groups. These results suggest that pALG is an effective treatment for SAA. Independent factors that influenced prognosis were VSAA, time from diagnosis to treatment of more than 30 d, and no response at 6 months.

Most previous studies have focused on hATG and rATG; however, studies comparing pALG and rATG, which are widely used in China, are lacking. We retrospectively analyzed the efficacies, survival, and safety profiles in patients that were treated with pALG and rATG, and found that the 1-yr overall response rate was 83.78% in the pALG group and the median time to first response was 94 (17–265) d, which are in agreement with the findings of Bing *et al.* (16) and Zhang *et al.* (19). The 5-yr overall survival rate of the patients was 82.22% and the event-free survival rate was 71.11% in the pALG group in the present study, while for the rATG group, our results showed that the 1-yr overall response rate was 66.67%, the median time to first response was 146 (31–334) d, and the 5-yr overall survival rate and event-free survival rate were both 68.75%, which were in accordance with the findings of the EBMT (20), the National Institutes of Health (NIH) (21), the Cleveland Clinic study (22), and Zheng *et al.* (2006) (23).

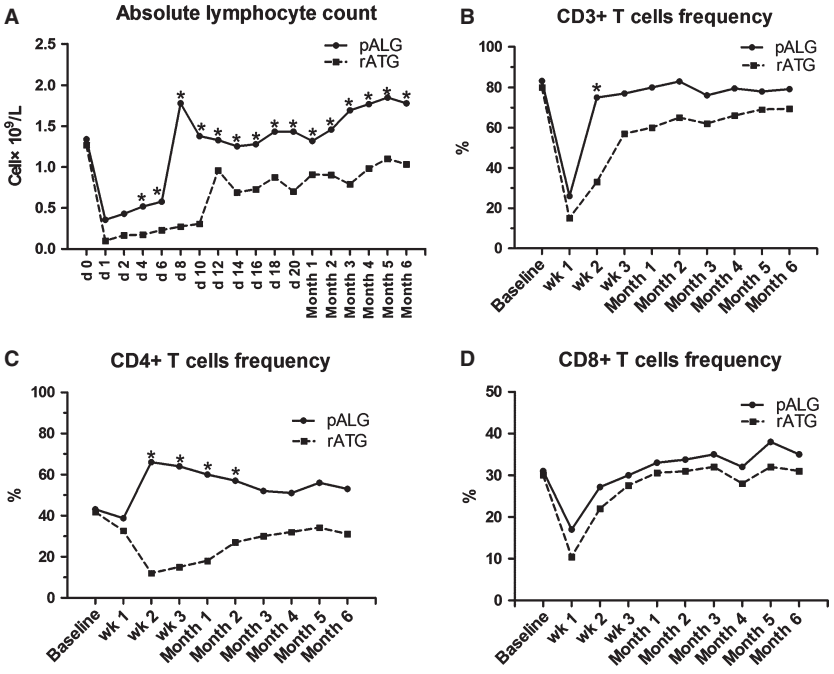

Figure 2 Changes in lymphocytes. Analysis of the lymphocyte counts in the two groups of patients: pALG group treated with porcine antilymphocyte globulin, and rATG group treated with rabbit antithymocyte globulin from baseline before the treatment to 6 months after the treatment. A: Absolute lymphocyte count. B: Percentage of CD3+ T cells. C: Percentage of CD4+ T cells. D: Percentage of CD8+ T cells. *Indicates a significant difference between the groups ($P < 0.05$).

These results showed that the 1-yr effective rate and time to response were significantly better in the pALG group than in the rATG group. Zheng *et al.* (1999) (24) found that the efficacy was poorer in the pALG group than in the rATG group. However, in that study, the patients in the pALG group were treated with pALG without CSA, while CSA treatment was performed for the patients in the rATG group. As the efficacy of application of ATG/ALG without CSA is not as good as the efficacy of combined application (9), their findings do not suggest that the efficacy of pALG is poorer than that of rATG. In the present study, no significant differences were found in the age, sex, severity of the disease, causes of the disease, pretreatment hemogram, and the time from diagnosis to treatment between the two groups, and both of the groups received identical CSA treatments, which ensured the influence of CSA on the efficacy was equal for both groups. In a study by Liu *et al.* (25), the 1-yr response rate was 69.05% in the pALG group, which was not significantly different compared with the rATG group. In the present study, the 1-yr response rate in the pALG group was substantially higher, which could be associated with the younger age, lower VSAA percentage, and longer time from diagnosis to treatment.

We showed that the 5-yr survival rate was better in the pALG group than in the rATG group, but the difference was not statistically significant. Previous studies have shown that the 6-month response rate is strongly associated with the requirement of blood transfusion and the long-term prognoses (8). The multivariate analysis of the factors affecting the survival in this study also showed that the risk of death within 5 yr after the treatment in the patients with no response at 6 months was 2.55-fold greater than the risk in the responders; however, although the response rate at 6 months was significantly lower in the rATG group than in the pALG group, the 5-yr survival rate was not significantly different between these two groups. As the overall survival rate and event-free survival rate in the pALG group were higher than those in the rATG group, we speculate that the lack of statistical significance could be associated with the small sample size in the rATG group.

We found that lymphocyte count decreased in both groups, while the rATG group had a longer time period of lymphocyte decrease and lower lymphocyte counts. We then further compared the changes in subsets of T cell until 6 months after the treatment, and found that the inhibitory effects on the CD8+ T cells were not significantly different between these two groups, while the inhibitory effects on CD4+ T cells were significantly higher in the rATG group than in the pALG group. Therefore, we concluded that rATG demonstrated stronger immunosuppressive effects than pALG, and the effects were mainly on CD4+ T cells. Appropriate immunosuppressive effects for AA therapy involve decreasing the activated CD8+ T cells to eliminate the negative-regulatory effects of abnormal cellular immunity on medullary hematopoiesis. However, if the lymphocytes decrease too much and for a long period of time, and especially if CD4+ T cells are depleted, which have regulatory effects and stimulate medullary hematopoiesis (26, 27), bone marrow cannot then be stimulated to restore hematopoiesis, resulting in poor efficacy (21). Therefore, we speculate that the mechanisms that caused the differences in the efficacies between these two treatments could be a decrease of CD8+ T cells, which occurred in both groups, resulting in the effectiveness of pALG and rATG, while the decrease in

CD4+ T cells caused the suboptimal effects in the rATG group. In addition, depletion of CD4+ T cells could also be one reason why rATG shows good efficacy in treating graft-vs.-host disease after kidney transplantation (28, 29).

These results could have a major impact on treatment of SAA in China where pALG is already commonly used. Currently, rATG is twice as expensive as pALG. This study provides evidence that the less expensive treatment option is not inferior and may be superior to the more expensive treatment.

In summary, the findings of the present study showed that pALG had better efficacy and similar survival, and safety profiles compared with rATG. The efficacy of pALG could be associated with pALG being able to stimulate the bone marrow to restore hematopoiesis more quickly. As a drug developed in China, pALG has only been investigated by a few previous studies; thus, the present study is of important value in guiding the clinical application of this drug, as well as providing evidence for further investigations. Further prospective clinical trials and experimental studies are needed to investigate the exact mechanisms of the effects of ATG/ALG from different species.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Treatment strategies.

Appendix S2. Causes of death.

Appendix S3. Patient survival.

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临床研究

猪抗人淋巴细胞球蛋白与兔抗人胸腺细胞球蛋白治疗重型再生障碍性贫血的疗效比较

杨 楠,马肖容,张 卉,曹星梅,陈银霞,何爱丽,刘 捷,赵万红,张王刚
西安交通大学医学部第二附属医院血液内科,陕西 西安 710004

摘要:目的 比较猪抗人淋巴细胞球蛋白(ALG)与兔抗人胸腺细胞球蛋白(ATG)治疗重型再生障碍性贫血(再障)的疗效。**方法** 回顾分析2004~2013年接受免疫抑制治疗的重型再障患者(猪ALG组43例,兔ATG组32例)6个月有效率,χ^2检验比较组间差异。Kaplan-Meier法绘制生存曲线,log-rank检验比较组间差异。**结果** 猪ALG组6个月有效率为79.07%,高于兔ATG组(56.25%)且差异显著(P=0.034);5年总生存率为86.047%,高于兔ATG组(72.878%),但差异不显著(P=0.190)。**结论** 猪ALG血液学应答优于兔ATG,预后和安全性相似。

关键词:猪抗人淋巴细胞球蛋白;兔抗人胸腺细胞球蛋白;再生障碍性贫血

Therapeutic effects of porcine versus rabbit antithymocyte globulins for treatment of severe aplastic anemia

YANG Nan, MA Xiaorong, ZHANG Hui, CAO Xingmei, CHEN Yinxia, HE Aili, LIU Jie, ZHAO Wanhong, ZHANG Wanggang
Department of Hematology, Second Affiliated Hospital, Xi'an Jiaotong University Health Science Center, Xi'an 710004, China

Abstract: Objective To compare the efficacy of porcine and rabbit antithymocyte globulins (ATG) in the treatment of severe aplastic anemia (SAA). **Methods** We reviewed the clinical data of 43 SAA patients receiving porcine ALG treatment and 32 patients receiving rabbit ATG treatment between 2004 and 2013 in our hospital. The overall response rates of the patients at 6 month were compared, and the patients' survival in the two groups was analyzed using Kaplan-Meier survival curves. **Results** The overall response rates at 6 months was significantly higher in porcine ALG group than in rabbit ATG group (79.07% *vs* 56.25%, P=0.034). The 5-year overall survival was also higher in porcine ALG group than in rabbit ATG group, but this difference was not statistically significant (86.047% *vs* 72.878%, P=0.190). **Conclusions** Porcine ALG is superior over rabbit ATG in terms of hematological response but is comparable with rabbit ATG in view of the patients' survival and safety.

Key words: porcine antilymphocyte globulin; rabbit antithymocyte globulin; aplastic anemia

再生障碍性贫血(再障)是一种细胞免疫异常引起造血干/祖细胞及外周血三系细胞减少的获得性骨髓衰竭性疾病[1]。免疫抑制疗法(IST)是一种基于抗人胸腺/淋巴细胞球蛋白(ATG/ALG)联合环孢素A(CSA)的治疗。它适应于缺少人白细胞抗原相合同胞供者的重型再障患者[2-3],可使2/3的患者受益[4-6]。

目前认为ATG/ALG治疗再障的机制是清除循环池中异常活化的T细胞[7]。补体依赖的细胞溶解或活化相关的凋亡作用是其主要清除途径。除此之外,近期大量研究表明ATG/ALG对免疫系统还有多种调节功能:(1)调节细胞表面分子介导的白细胞/内皮细胞相互作用;(2)诱导B细胞系凋亡;(3)干预树突状细胞功能以及(4)诱导产生调节性T细胞及自然杀伤性T细胞。

根据免疫动物的不同,ATG/ALG分为马ALG、兔ATG及猪ALG[8]。迄今为止,对于ATG/ALG种属选择尚无定论。目前国内可获得猪ALG和兔ATG治疗重型再障。由于Chen[9]的研究猪ALG总应答率达74.5%,且其治疗费用仅为兔的1/3,越来越多的临床医生将猪ALG作为首选。但是这多为经验性用药,缺乏临床试验支持。因此,本研究对2004~2013年在我科接受猪ALG的43例及兔ATG 32例重型再障患者进行回顾性分析,比较两种ATG/ALG在6个月血液学应答、5年生存及安全性方面的数据,为临床工作中ATG/ALG选择及进一步探讨其治疗机制提供依据。

1 资料和方法

1.1 资料

1.1.1 病例的纳入与剔除 研究对象是2004~2013年在

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作者简介:杨 楠,博士研究生,E-mail: Shmily1989-2008@163.com;马肖容,博士研究生,副主任医师,E-mail: maxro910@163.com。杨 楠、马肖容共同为第一作者
通信作者:张王刚,教授,主任医师,E-mail: ZhangW_G@163.com

西安交通大学医学院第二附属医院血液内科接受IST的75例(猪ALG组43例,兔ATG组32例)重型再障患者。再障诊断及分度分型严格按照指南[10]。纳入患者在治疗前均未接受骨髓移植或IST。纳入患者年龄≥4岁。剔除标准:存在(1)骨髓增生异常综合征的临床证据;(2)阵发性睡眠性血红蛋白尿症(PNH)特征性溶血或其相关的血栓病史,流式细胞仪检测PNH克隆大于50%;(3)严重的、不能控制的感染或不能解释的、体温>38 ℃的发热。

本研究获得我院医学专业学术委员会和伦理委员会批准。在参加本研究前,均取得患者及/或其法定监护人的知情同意。

1.1.2 *随访* 第一随访终点是治疗后6个月有效,第二随访终点是2014年12月生存或死亡。治疗后6月每2~3天行血常规1次,6~12月每周1次,1年后每月1次。治疗后1月每周行T细胞亚群检测1次,6月内每月1次。治疗后3月行骨髓穿刺及活检检查、染色体核型、GPI-AP缺失1次,如情况稳定每半年复查1次,如随访期间血常规出现异常立即复查。上述实验室检查用以观察血象应答、细胞免疫变化及有无复发或转化。

1.2 *治疗方案与评价标准*

1.2.1 *治疗方案* 猪ALG组:猪ALG(武汉生物制品研究所),25 mg/(kg·d),静滴,连用5 d;兔ATG组:兔ATG(Thymoglobulin, Genzyme),2.5 mg/(kg·d),维持12~18 h,静滴,连用5 d。为预防变态反应,ATG/ALG注射前口服抗组胺剂。为预防血清病,第1~15天,泼尼松1 mg/(kg·d)静滴,第16天起减量,每5 d减为原量的一半,第30天停药。第6天起,CSA(诺华制药公司)成人3 mg/(kg·d),儿童5 mg/(kg·d),2~3次/d,口服,维持血CSA谷浓度为150~250 ng/mL,持续6~12个月,后在三系细胞稳定的情况下逐渐减量。所有患者在雄激素、造血生长因子及输血,抗感染及其他支持治疗上无差异。

1.2.2 *评价标准* 疗效是通过至少间隔4周的2次或2次以上血常规确定,患者在采血时已停用造血生长因子至少1周。评价标准严格按照指南[10]。

1.3 *统计学处理方法*

所有结果采用SPSS18.0统计软件分析。组间计量资料比较采用*t*检验或Mann-Whitney非参数检验;组间率比较采用Pearson χ^2检验或连续性校正χ^2检验;生存曲线采用Kaplan-Meier法,组间比较采用log-rank检验。预后多因素分析采用Cox回归分析法。*P*值均取双侧,且当*P*<0.05时,认为差异有统计学意义。

2 结果

2.1 *患者特征*

两组患者的基本临床特征组间无显著性差异(*P*>0.05,表1)。猪ALG组随访中位时间为34.5(0.3~103)月,兔ATG组为26(0.37~125)月,组间无显著差异(*P*>0.05)。所有患者随访至第1终点,73例患者随访至第2终点,失访2例(2.67%),失访患者至最后一次随访均持续无效。PNH克隆阳性指GPI-AP细胞阳性率小于<95%。仅2012年后治疗的部分患者行PNH克隆(13例猪ALG,12例兔ATG)和染色体(16例猪ALG,14例兔ATG)检查。CSA治疗时间指初次获得最佳疗效至停药的间隔时间。

2.2 *血液学应答*

2.2.1 *6个月内有效率* 早期死亡(治疗后90 d内死亡)患者被认为IST无效。猪ALG组6个月有效率为79.07%,高于兔ATG组(56.25%),有统计学意义(*P*=0.034,表2)。此外,猪ALG组初次获得有效的中位时间为94(17~265)d,短于兔ATG组146(31~334)d,差异有统计学意义(*P*<0.05)。

2.2.2 *血象恢复情况* 猪ALG组与兔ATG组ANC>1.0×10^9/L的中位时间分别为12(0~27)d、16(0~36)d,差异不显著(*P*=0.87);脱离红细胞输注为35(0~131)d、56(27~167)d,无统计学意义(*P*=0.62);脱离血小板输注为82(16~265)d、106(29~334)d,也无明显差异(*P*=0.41)。

2.3 *生存情况*

2.3.1 *5年生存率* 猪ALG组5年总生存率为86.047%,高于兔ATG组的72.878%,但差异不显著(*P*=0.1899);无事件生存率(除发生转化或复发的生存患者)为74.118%,与兔ATG组(72.878%)相近(*P*=0.8621)。至第2随访终点,1例猪ALG和4例兔ATG患者仍未达到有效,这些患者未行第2次IST或骨髓移植术,均依赖输血存活(图1)。

2.3.2 *死亡病例及死因* 猪ALG组与兔ATG组的早期死亡患者均为5例,早期死亡率为11.63% *vs* 15.63%(*P*=0.873);总死亡患者分别为6例和8例,死亡率为13.95% *vs* 25%(*P*=0.225)。所有死亡患者未达有效。感染是其主要死因,85%死亡病例与感染直接相关(表3)。

2.3.3 *复发* 至第2随访终点,兔ATG组未观察到复发;猪ALG组有3例(6.98%,*P*=0.353)患者分别于治疗的第147、283、540天复发。3例患者均未行第2次IST或骨髓移植术,通过延长CSA用药,分别于第262、570、433天再次获得部分缓解,其中2例最终获得完全治愈。

2.3.4 *转化* 至第2随访终点,兔ATG组未观察到转化;猪ALG组有2例发生转化,组间转化率无显著差异(*P*=0.609)。其中,1例于第125天诊断为骨髓增生异常综合征伴5q31缺失;1例于第455天诊断为PHN。2例患者在转化前均未达有效,依赖输血存活。

表1 患者临床基本特征

Tab.1 PBaseline clinical characteristics of the patients

Baseline Characteristics	pALG	rATG	*P*
n	43	32	
Median follow-up (months)	34.5 (0.3-103)	26 (0.37-125)	0.18
Gender (male/female)	13/30	19/13	0.61
Median age (year)	21 (4-56)	24.5 (7-64)	0.46
Age (<40/≥40)	36/7	26/6	0.78
Severity (VSAA/SAA)	28/15	13/19	0.35
Type (Ⅰ/Ⅱ)	33/10	20/12	0.18
Etiology (idiopathic/post-hepatitis)	41/2	32/0	0.61
Blood counts			
Ret ($\times10^9$/L)	7.3 (0-18.9)	11.3 (0-19.8)	0.53
Ret (%)	0.12 (0.03-1.73)	0.16 (0.07-1.52)	0.34
ANC ($\times10^9$/L)	0.24 (0.02-1.03)	0.37 (0-1.0)	0.98
PLT ($\times10^9$/L)	13 (2-20)	11.5 (6-20)	0.89
ALC ($\times10^9$/L)	1.34 (0.14-2.6)	1.44 (0.42-2.76)	0.75
ALC (%)	64.54 (18.9-97.04)	64.16 (22.1-94.9)	0.65
PNH clone	0	0	
Abnormal cytogenetic clone	0	0	
Previous treatment			
Yes	4 (9.30%)	4 (12.5%)	0.95
Androgen±CSA	3	4	
IVIg±corticosteroids	1	0	
Whole blood CSA levels at 6 months	178±47.4	193±72.3	0.43
Median CSA treatment duration (days)	632 (153-1284)	533 (106-1312)	0.84

Ret: Reticulocyte; ANC: Absolute neutrophil count; PLT: Platelet; ALC: Absolute lymphocyte count.

表2 两组血液学应答率比较

Tab.2 Comparison of overall response rate

Hematological Response		pALG	rATG	*P*
1 month	CR	0	0	-
	PR	4 (9.30%)	1 (3.13%)	0.553
	OR	4 (9.30%)	1 (3.13%)	0.553
3 months	CR	3 (6.98%)	3 (9.38%)	1.000
	PR	21 (48.84%)	9 (28.12%)	0.070
	OR	24 (55.82%)	12 (37.50%)	0.116
6 months	CR	9 (20.93%)	6 (18.75%)	0.815
	PR	25 (58.14%)	12 (37.50%)	0.077
	OR	34 (79.07%)	18 (56.25%)	0.034*

CR: Complete response; PR: Partial response; OR: Overall response. *P< 0.05.

2.4 预后多因素分析

多因素分析显示，性别、年龄、治疗前血象、IST前治疗及ATG/ALG种属差异并不影响预后，而极重型再障、重Ⅱ型再障及6个月无应答是预后的独立危险因素（表4）。其中，极重型患者5年死亡率是重型的2.5倍（P=0.04，95%可信区间1.03~5.68）；重Ⅱ型患者是重Ⅰ型的3.6倍（P=0.01，95%可信区间1.50~10.10）；6个月无应答患者是有应答的3.75倍（P=0.01，95%可信区间1.03-6.04）。

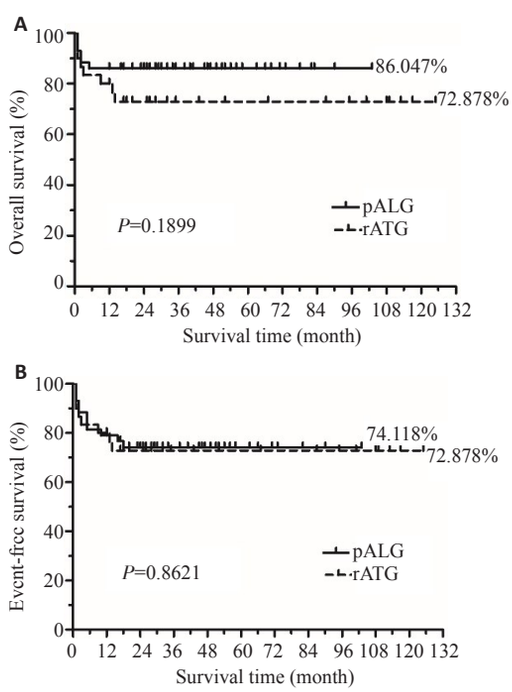

图1 两组生存曲线比较

Fig.1 Comparison of survival curves. There was no significant difference (P>0.05) in the overall survival (*A*) or event-free survival (*B*).

表3 2组死因分析

Tab.3 Cause of death of the patients in the two groups

Cause of death (15)	pALG	rATG
Sepsis (6)	d9/d26/d125*	d11/d17/d27
Pneumonia bacterial infection (2)	d41	d245
Invasive fungal infection (4)	d33*	d43/d84
Intracranial hemorrhage (1)	d27	-
Cardiac arrest with sepsis (1)	-	d387
Multiorgan failure with sepsis (1)	-	d403

*Post-hepatitis AA.

2.5 不良反应与毒性

两组变态反应、血清病、感染、出血、肝肾功能损害、高血压、糖耐量异常或空腹血糖受损发生率均无明显差异(P>0.05,表5)。所有出现的不良反应在给予对症治疗后恢复,不良反应轻微而可控,未出现治疗相关死亡。

2.6 淋巴细胞计数变化

为探讨猪ALG与兔ATG疗效差异的机制,我们绘制了IST前后外周血淋巴细胞计数变化曲线(图2)。IST前两组患者淋巴细胞绝对计数及$CD8^+$ T细胞百分百无统计学差异。治疗后第6、8、10、14天及第1~6个月,猪ALG组淋巴细胞绝对计数均大于兔ATG组,且差异显著(P<0.05);而$CD8^+$ T细胞百分百始终无显著差异(P>0.05)。

表4 预后的多因素分析

Tab.4 Analysis of factors associated with overall survival at 5 years after IST

Clinical characteristics	P	OR (95% CI)
Male	0.97	1.03(0.13-7.74)
Age≥40 years	0.87	0.92(0.27-2.87)
VSAA	0.04*	2.50(1.03-5.68)
Blood counts HB<60 g/L	0.35	1.77(0.52-6.01)
ANC<0.5×10^9/L	0.07	2.47(0.91-6.68)
PLT≤10×10^9/L	0.47	1.53(0.47-4.92)
Type Ⅱ SAA	0.01*	3.60(1.50-10.10)
Have previous treatment	0.34	1.49(0.65-3.40)
pALG *vs* rATG	0.32	0.63(0.25,1.58)
NR at 6 months	0.01*	3.75(1.03-6.04)

IST: Immunosuppression therapy. *P<0.05.

表5 不良反应与毒性

Tab.5 Side effects and toxicity

Side Effects	pALG	rATG	P
Allergy	5	3	1.00
Serum sickness	17	12	0.86
Fever	10	9	
Rash	3	1	
Arthralgia	4	2	
Infection	30	21	0.70
Hemorrhage	6	4	1.00
Hypertension	7	3	0.60
Diabetes mellitus	2	1	1.00
Elevated liver function tests	10	5	0.41
Renal impairment	3	0	0.35
Other	1	2	0.79

3 讨论

对于缺少人白细胞抗原相合同胞供者的重型再障患者,基于ATG/ALG联合CSA的IST是其一线治疗方案[2-3]。自2011年起,发表的大量临床研究表明[12-14],不同种属来源的ATG/ALG疗效存在差异。因此,临床医生对于种属剂型的决策可能影响到再障患者的预后。目前,除兔ATG外,我国临床医生普遍使用猪ALG治疗重型再障,但是文献中有关猪ALG疗效的报道极少。基于上述原因,比较猪ALG与兔ATG在再障临床治疗

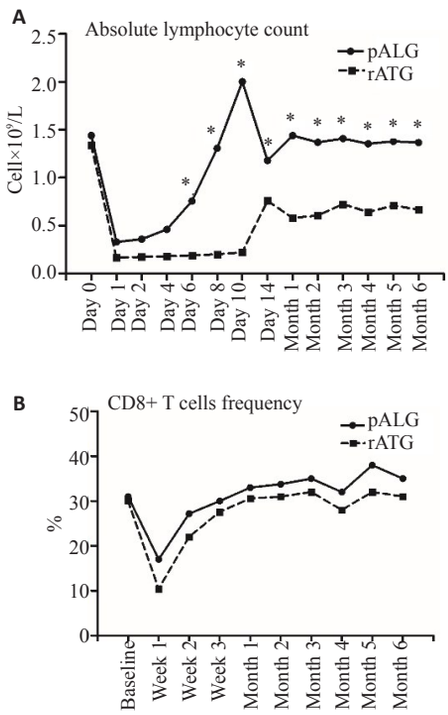

图2 外周血淋巴细胞计数变化曲线

Fig.2 Kinetics of lymphocyte count after each ATG administration. *A*: Absolute lymphocyte count; *B*: $CD8^+$ T cell frequency. *P<0.05.

中的疗效具有重要意义。

本研究回顾性比较猪ALG与兔ATG的疗效，得到如下结果：猪ALG组与兔ATG组6个月有效率分别为79.07%和56.25%，起效中位时间分别为94（17~265）d和146（31~334）d。其中，猪ALG的应答情况与国内Chen[9]及Wei等[11]报道结果基本一致，而兔ATG也同EBMT[12]、NIH[13]、Cleveland诊所[14]及国内Zhang等[15]报道结果相近。两组比较显示，猪ALG 6个月有效率及起效中位时间均明显优于对照组。但是在唯一可获得的同类文献中，Liu等[16]报道两组应答率并无明显差异。分析两项研究结果，我们发现本研究中兔ATG组的应答与Liu研究相近，而猪ALG组疗效优于其研究。为排除选择偏倚及实施偏倚，我们进一步比较了两项研究的样本基线资料及治疗策略。两项研究在患者年龄、极重型及重Ⅱ型再障(37.50%)比例上基本相近，然而Liu使用ALG剂量[30 mg/(kg·d)]高于本研究[25 mg/(kg·d)]。既往研究表明，由于免疫抑制作用加重，ATG大剂量组疗效低于中等剂量组[17]，因此我们猜想这种剂量差异是导致疗效结论不同的原因。

对两组患者的生存情况分析，我们发现猪ALG组5年总生存率为86.047%，无事件生存率为74.118%，兔ATG组均为72.878%。组间比较表明，猪ALG组5年生存情况优于兔ATG组，但差异不显著。为了明确组间差异是否受其他混杂因素影响，我们进一步进行了预后的多因素分析。Cox回归分析显示，兔ATG并不是5年累计生存率的独立危险因素。综合上述单因素及多因素分析结果，我们得出：猪ALG组与兔ATG组患者的生存情况近似。此外，预后的多因素分析还表明，极重型、重Ⅱ型再障及6个月对治疗无反应是其独立危险因素，这与既往同类研究结果一致[12-14]。

此外，我们还对其治疗机制进行了初步探究。通过组内纵向比较发现，治疗后两组淋巴细胞绝对计数均较前下降。体内外研究显示[7]，通过补体依赖的细胞溶解作用介导循环池中T淋巴细胞的清除是ATG/ALG最重要的治疗机制。两组患者较治疗前淋巴细胞均明显降低，说明猪ALG和兔ATG治疗再障有效。且T细胞亚群分析显示两组间$CD8^+$ T细胞作用相似更进一步支持了上述结论。然而，组间横向比较发现，兔ATG组淋巴细胞缺乏的时间更持久，绝对数值更低。这表明：兔ATG对于非$CD8^+$ T细胞（如Treg细胞）的作用强于猪ALG。既往观察到的兔ATG对于移植物抗宿主病有更好的疗效，病毒感染发生率较高[17-19]；药物代谢动力学研究观察到兔ATG体内清除速率慢，治疗后1月的兔ATG浓度仍具有捕获T细胞的能力[20]：这些研究都从不同方面支持兔ATG有更强更持久的免疫抑制活性。于是，我们推测：不同程度的免疫抑制活性可能是疗效差异的重要机制。如果说，适当的免疫抑制消除活化$CD8^+$ T细胞对骨髓造血的负调节；那么，过度过长的淋巴细胞缺乏可能阻碍骨髓恢复并且增加感染发生率。

综上所述，在生存情况和安全性上，猪ALG与兔ATG相似，在血液学应答上优于兔ATG。机制可能与猪ALG免疫抑制适度，促进骨髓造血恢复有关。这为临床工作中ATG/ALG种属选择提供依据。但由于方法学限制可能受到偏倚影响，要得到更加可靠的结论仍需多中心随机对照双盲试验。

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（编辑：经 媛）

·论著·

猪抗人淋巴细胞球蛋白治疗重型再生障碍性贫血的临床研究

杨云　张王刚　曹星梅　陈银霞　何爱丽　刘捷　马肖容　赵万红　王剑利

［摘要］ **目的**　探讨一线应用猪抗人淋巴细胞球蛋白(P-ALG)联合环孢素(CsA)治疗重型再生障碍性贫血(SAA)的疗效及不良反应。**方法**　回顾性分析我科应用 P-ALG 联合 CsA 治疗 36 例 SAA 患者的疗效、并发症及转归,并对患者的年龄、疾病严重程度、发病距应用 P-ALG 的时间等因素与疗效进行相关分析。**结果**　36 例患者中死亡 4 例,白细胞恢复中位时间为 30 天,脱离红细胞输注的中位时间为 55 天,脱离血小板输注的中位时间为 60 天。1 年总有效率为 86.2%,2 年总有效率达 91.7%,1 年总有效率与性别、年龄、疾病分型、CD8 比例无明显相关($P>0.05$),病程 <3 个月及外周血中性粒细胞(ANC)$\geq 0.2\times10^9$/L 患者的有效率高;与国内及欧美国家报道的利用马或兔抗胸腺细胞球蛋白(ATG)治疗 SAA 的有效率相当,而不良反应小,花费仅为马或兔 ATG 治疗的1/3 ~ 1/2。**结论**　应用 P-ALG 联合 CsA 治疗重型再生障碍性贫血疗效显著,不良反应轻微。

［关键词］ 再生障碍性贫血；　猪抗人淋巴细胞球蛋白；　环孢素；　疗效

Clinical study of combination of pig antithymocyte globulin and cyclosporine A as first-line therapy for adult severe aplastic anemia　*YANG Yun, ZHANG Wanggang, CAO Xingmei, et al. Department of Hematology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an* 710004, China

［**Abstract**］ **Objective**　To analyze the efficacy and side effects of combination of pig antithymecyte globulin(ALG) and cyclosporine A(CsA) as the first-line immunesuppressive therapy(IST) for adult severe aplastic anemia(SAA). **Methods**　The efficacy, complications, therapeutic response relevant factors were analysed for thirty-six adult SAA patients treated with pig ALG and CsA in our hospital. **Results**　Of thirty-six patients, four died. The median time of the white blood cell recovery, to transfusion of red blood cell independent, and to transfusion of palete independent was 30, 55 and 60 days respectively. The overall response rate of one year was 86.2%. The 2-year overall response was 91.7%, and the overall response rate for a year was not related to sex, age, subtypes, and the ratio of CD8($P>0.05$). Patients with course of disease less than three months or peripheral blood absolute neutrophil count(ANC) $\geq 0.2\times10^9$/L had a higher response rate. The response rate was close to horse or rabbit ATG for SAA as reported in our country, Europe and United States, but with less side effects and only 1/3-1/2 expense. **Conclusion**　Pig-ALG in combination with CsA first-line for adult severe aplastic anemia leads to excellent treatment outcomes with minor side effects.

［**Key words**］ Aplastic anemia;　Pig antithymocyte globulin;　Cyclosporine A;　Treatment effect

重型再生障碍性贫血(SAA)是一种获得性骨髓造血功能衰竭,以全血细胞减少、贫血、出血、感染为主要特点,预后较差。免疫抑制治疗(IST)是不宜进行造血干细胞移植 SSA 患者的主要治疗方案,联合应用抗胸腺细胞球蛋白(ATG)及环孢素(CsA)是目前公认的标准免疫抑制治疗方法,其有效率为 60% ~80%[1-2]。进口马或兔 ATG 治疗费用昂贵,猪抗人淋巴细胞球蛋白(P-ALG)治疗 SSA 疗效显著,花费相对较低。我们对 2007 年以来在我科应用P-ALG联合 CsA 治疗的 36 例不能进行造血干细胞移植 SAA 患者的临床资料进行分析,以探讨其临床应用价值。

对象与方法

1. 对象:我科 2007 年 1 月 ~2012 年 3 月收治的 SSA 患者 36 例,治疗前均明确诊断及分型,经仔细询问病史、体格检查和常规细胞遗传学检查、Ham 试验及外周血粒细胞和成熟红细胞流式细胞术检测 CD55、CD59 表达,所有病例均符合 SAA 诊断标准,排除急性造血功能停滞、骨髓增生异常综合征、阵发性睡眠性血红蛋白尿、骨髓纤维化、免疫性三系细胞减少、范科尼贫血等疾病,诊断标准参照《血液病诊断及疗效标准》[3]。肝炎相关性再生障碍性贫血(AA)定义为与肝炎同时或在肝炎患病 6 个月内发生的 AA,血清丙氨酸

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作者单位:710004 西安交通大学第二附属医院血液科

转氨酶升高至少达 200 U/L。36 例患者中，男 23 例，女 13 例，年龄 14 ~ 72 岁，中位年龄 41 岁，病程 2 周 ~ 2 年（中位病程 2 个月），诊断 SAA Ⅰ型 13 例，SAA Ⅱ型 23 例，血红蛋白中位值为 55（40 ~ 100）g/L，中性粒细胞（ANC）中位值为 0.35（0.17 ~ 0.49）$\times 10^9$/L，血小板中位值为 6（1 ~ 19）$\times 10^9$/L，网织红细胞中位值为 5（2 ~ 18）$\times 10^9$/L。其中极重型再生障碍性贫血（VSAA）10 例，肝炎相关 AA 3 例。

2. 方法

（1）应用药物：P-ALG（规格：5 ml：每瓶 250 mg）；CsA（规格：25 mg × 40 粒）；康力龙（规格：2 mg × 100 片）；促红细胞生成素（规格：每支 10 000 IU）；重组人粒细胞刺激因子（规格：每支 100 μg）。

（2）治疗方案：接受 IST 的 SAA 或 VSAA 患者均无 HLA 相合同胞供者，或拒绝接受造血干细胞移植治疗。所有患者均入住无菌层流病房，静脉滴注 P-ALG（每天 20 ~ 30 mg/kg，d1 ~ 5），CsA（每天 3 ~ 5 mg/kg，分两次口服，每 12 小时 1 次），根据血药浓度调整剂量，使 CsA 谷浓度为 150 ~ 250 μg/L，所有患者均在获得最佳血液学治疗反应后，继续应用 CsA，1 年后开始减量。一般疗效达平台期后持续服药至少 12 个月。所有患者应用 P-ALG 时均静脉滴注地塞米松（5 ~ 10 mg/d）预防血清病及过敏反应，后改为泼尼松口服并逐渐减量，疗程 1 个月。同时所有患者口服雄激素康力龙每日 6 mg 或十一酸睾酮（每日 60 ~ 80 mg），治疗期间监测血常规及肝肾功能。所有患者均联合应用重组人粒细胞刺激因子及促红细胞生成素。

（3）疗效标准：参照我国现行的再生障碍性贫血疗效标准[3]，疗效分为基本治愈、缓解、明显进步和无效，前 3 种均视为有效。SAA 患者 IST 治疗后临床复发是指 IST 获得缓解后，出现任一系列的外周血细胞计数下降至缓解期中位水平的 50% 以下，或需要血液制品输注，或再次降至 SAA 诊断水平。

（4）随访：定期复查血常规、肝肾功能、CsA 血药浓度、骨髓检查、CD55、CD59 以及骨髓染色体核型了解有无晚期并发症。

3. 统计学处理：应用 SPSS 11.0 软件进行统计分析。率的比较采用连续校正 χ^2 检验及 Fisher 确切概率法，Kaplan-Meier 评估法绘制生存曲线。

结　果

1. 疗效分析：36 例患者白细胞恢复中位时间为 30 天，脱离红细胞输注中位时间为 55 天，脱离血小板输注中位时间为 60 天。按照不同时间点评价疗效，3 个月缓解率为 5.56%，明显进步率为 36.1%，总有效率为 41.7%；6 个月缓解率为 25.0%，明显进步率为 44.4%，总有效率为 69.4%；1 年基本治愈率为 50.0%，缓解率为 30.6%，明显进步率为 5.6%，总有效率为 86.2%；2 年基本治愈率为 88.9%，达到缓解率为 2.8%，明显进步率为 0，总有效率为 91.7%；其中死亡 4 例，1 例于接受 IST 治疗后 30 天死于巨细胞脑炎（CMV）脑炎，另 1 例于接受 IST 治疗后 45 天死于肺部感染，其余 2 例分别于接受 IST 治疗后 4 个月 21 天及 20 个月死于出血和感染，占所有接受治疗患者的 11.11%。3 例肝炎相关 AA 患者，2 例于接受 IST 治疗后 6 个月获得缓解，1 例死亡。

2. 治疗效果与患者临床病理特征的关系：见表 1。结果显示，1 年的有效率与患者性别、年龄、疾病分型、CD8 比例及网织红细胞计数无明显相关（$P > 0.05$），而病程≥3 个月患者的有效率为 50.0%，病程 < 3 个月患者的有效率为 96.7%，差异有统计学意义（$P < 0.05$），而且治疗前外周血 ANC ≥ 0.2×10^9/L 患者的有效率（88.5%）明显高于 ANC < 0.2×10^9/L 患者的有效率（40.0%，$P < 0.05$）。

表 1　患者疗效与临床病理特征的关系

临床病理特征		例数	有效率（%）	*P* 值
性别	男	23	91.3	0.609
	女	13	84.6	
年龄	≥20 岁	26	88.5	1.000
	<20 岁	10	90.0	
分型	SAA Ⅰ型	13	76.9	0.124
	SAA Ⅱ型	23	95.7	
病程	≥3 个月	6	50.0	0.010
	<3 个月	30	96.7	
外周血 ANC	≥0.5×10^9/L	20	85.0	0.245
	<0.5×10^9/L	16	62.5	
	≥0.2×10^9/L	26	88.5	0.010
	<0.2×10^9/L	10	40.0	
CD8	正常	24	91.7	0.588
	升高	12	83.3	
网织红细胞计数	正常	16	50.0	0.181
	<15×10^9/L	20	45.0	

3. 不良反应：患者出现发热 8 例（22.22%）、寒战 10 例（27.78%）、头痛 6 例（16.67%）、皮疹 5 例（13.89%）、心悸 5 例（13.89%）、关节疼痛 4 例（11.11%）、溶血 2 例（5.56%），明显肝功能损害 2 例（5.56%），肾功能损害 1 例（2.78%）。8 例（22.22%）出现血清病反应，均发生于开始输注 ALG 后 2 ~ 3 周，表现为发热、皮疹、口腔溃疡、肌肉和骨骼疼痛等。上述不良反应症状轻微，经适当处理后可有效控制，患者多能耐受。

4. 复发、生存率及克隆演变：在随访时间内，除 1 例失访、4 例死亡外，31 例目前生存患者的中位随访时间为 32 个月。36 例患者 3 年的累积生存率为 86.4%（图 1）。复发 1 例，转化为骨髓增生异常综合征 1 例，患者于治疗后 23 个月出现三系细胞下降，FISH 检测结果为 $5q^-$，未见并发急性白血病及阵发性睡眠性血红蛋白尿。

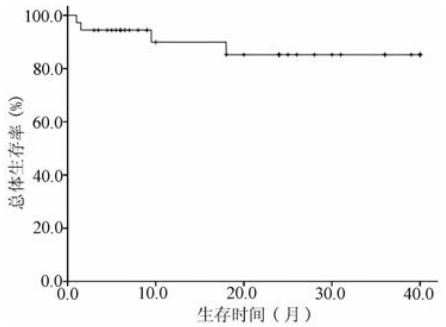

图 1　患者的生存率

讨　论

关于国产 P-ALG 联合 CsA 治疗重型再生障碍性贫血的研究国内外报道较少，本研究结果表明，P-ALG 联合 CsA 治疗重型再生障碍性贫血的 3、6 个月、1 年及 2 年有效率分别为 41.7%、69.4%、86.2% 及 91.7%，高于国内及欧美国家报道的利用马或兔 ATG 治疗 SAA 的有效率[4-6]，与邵英起等[7]应用国产 P-ATG 联合环孢素治疗 SAA 患者 3、6 个月疗效及 5 年总生存率均高于德国产兔 ATG 研究结果一致。曹慧敏等[8]对 76 例 SAA 一线应用兔 ATG 联合 CsA 免疫抑制治疗的有效率为 82.2%，获得治疗反应的患者脱离血制品输注的中位时间为 60（10 ~ 210）天，其中脱离红细胞输注中位时间为 55（0 ~ 210）天，脱离血小板输注中位时间为 49（2 ~ 166）天。本研究中白细胞恢复（ANC ≥ 1.0×10^9/L）中位时间为 30 天、脱离红细胞输注中位时间为 55 天，脱离血小板输注的中位时间为 60 天，与上述曹慧敏等[8]的研究结论一致。由此可见，与其他有关马或兔 ATG 疗效的研究相比，P-ALG 的有效率更高，起效时间相当。但由于本研究例数较少，仍需大样本量的临床研究进一步证实。另外，我们观察到，部分患者 3 个月以后起效，因此提示我们在临床工作中应鼓励患者坚持治疗，不要轻易停药。

对患者疗效与性别、年龄、分型、病程、外周血 ANC、CD8 比例及网织红细胞计数的分析表明，疗效与患者性别、年龄、分型、CD8 比例及网织红细胞计数无关，病程≥3 个月及外周血 ANC < 0.2×10^9/L 患者的疗效明显低于病程 < 3 个月及外周血 ANC ≥ 0.2×10^9/L 的患者，与国外 Hattori 等[9]的报道一致。其机制可能是 ALG 早期应用可以及时抑制 T 细胞及骨髓抑制因子对造血干细胞及造血微环境损伤，促进造血功能恢复；另外，因为患者粒细胞明显缺乏，延迟治疗合并严重感染发生率高，早期死亡率高，所以一旦诊断，应尽早治疗。

P-ALG 不良反应轻微，发热、寒战经给予糖皮质激素及异丙嗪均可有效控制，2 例患者应用 P-ALG 后发生溶血，表现为酱油色尿、血红蛋白下降、皮肤巩膜黄染，予以糖皮质激素、水化、碱化治疗后，患者溶血消失。从长期疗效看，36 例患者的早期死亡率及 3 年的生存率（86.4%）与文献报道一致，其中 1 例复发，复发患者考虑与 CsA 减量过快有关，加大 CsA 用量后三系细胞上升，表明国产 P-ALG 联合 CsA 对于初治 SAA 患者有较好且可靠的疗效。

本研究结果表明，P-ALG 联合 CsA 治疗 SAA 疗效显著，不良反应小，花费仅为进口马或兔 ATG 的 1/3 ~ 1/2，是不能进行造血干细胞移植重型再生障碍性贫血患者的首选治疗，值得临床推广应用。

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ORIGINAL ARTICLE

Efficacy of porcine antihuman lymphocyte immunoglobulin compared to rabbit antithymocyte immunoglobulin as a first-line treatment against acquired severe aplastic anemia

Limin Liu · Li Ding · Lin Hao · Xingxia Zhang · Xiaoli Li · Liansheng Zhang · Zhengdong Hao · Meiqing Lei · Yanming Zhang · Depei Wu

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Abstract Immunosuppressive therapy (IST) with antithymocyte immunoglobulin (ATG) or antilymphocyte immunoglobulin (ALG) and cyclosporine A (CsA) is the treatment of choice against severe aplastic anemia (SAA) worldwide. However, a comparison of the efficacy of porcine ALG (pALG) and rabbit ATG (rATG) as a first-line treatment for acquired SAA has not been reported. In the present study, we retrospectively analyzed SAA patients treated with either pALG (n=43) or rATG (n=32) and compared their hematologic responses and survivals. There were no significant differences in overall response (OR) rates between pALG and rATG groups at 3 months (OR 41.86 versus 40.62 % (P=0.914), 6 months (OR 66.67 versus 61.29 % (P=0.635), 9 months (OR 69.05 versus 61.29 % (P=0.490), or 12 months (OR 69.05 versus 64.51 % (P=0.684), respectively. The OR rates in patients with SAA or very severe aplastic anemia (vSAA) in both groups were similar after a 12-month treatment (pALG 74.07 versus 60.00 %, P= 0.550; rATG 70.00 versus 54.55 %, P= 0.640). Patients who experienced <30-day interval between diagnosis and treatment displayed higher OR rates (at 12 months) than those with intervals ≥30 days (pALG 83.33 versus 50.00 %, P= 0.021; rATG 87.50 versus 40.00 %, P= 0.006). There were no significant differences in 2-year overall survival (OS) between pALG (87.4±6.2 %) and rATG (83.2±7.8 %) (P=0.493). Infection was the major cause of death in both groups. In summary, pALG+CsA showed similar efficacy as rATG+CsA, as a first-line treatment for acquired SAA.

Keywords Porcine antihuman lymphocyte immunoglobulin · Immunosuppressive therapy · Severe aplastic anemia

Limin Liu, Li Ding, and Lin Hao contributed equally to this paper. They are co-first authors.

L. Liu · X. Li · D. Wu (✉)
The First Affiliated Hospital of Soochow University, Jiangsu Institute of Hematology, Key Laboratory of Thrombosis and Hemostasis of Ministry of Health, Collaborative Innovation Center of Hematology, Suzhou, Jiangsu Province 215006, People's Republic of China
e-mail: wudepei@medmail.com.cn

L. Liu · X. Zhang · Y. Zhang (✉)
Department of Hematology, Huai'an Hospital Affiliated to Xuzhou Medical College and Huai'an Second People's Hospital, 62 Huaihai South Road, Huai'an, Jiangsu Province 223002, People's Republic of China
e-mail: zhangyanming2005@126.com

L. Ding
The People's Hospital of Yinzhou, Yinzhou Hospital Affiliated to Medical School of Ningbo University, Ningbo, Zhejiang Province 315040, People's Republic of China

L. Hao
The People's Hospital of Peixian, Peixian, Jiangsu Province 221600, People's Republic of China

L. Zhang · Z. Hao
The Second Hospital of Lanzhou University, Lanzhou, Gansu Province 730000, People's Republic of China

M. Lei
Haikou People's Hospital, Haikou, Hainan Province 570208, People's Republic of China

Introduction

Severe aplastic anemia (SAA) is a life-threatening disorder characterized by peripheral blood pancytopenia and bone marrow hypoplasia [1]. In Europe and North America, the annual incidence of acquired aplastic anemia is approximately two cases per million individuals [2]. In East Asia, this figure is two to three times higher [2]. If left untreated, SAA is almost always fatal. For patients newly diagnosed with SAA or very severe aplastic anemia (vSAA) and less than 40 years of age, a matched sibling hematopoietic stem cell transplantation (HSCT) is considered as the first choice of therapy [3]. In contrast, patients who are older than 40 years of age or lack a matched sibling are often subjected to immunosuppressive therapy (IST) [3]. Although the survival rate following both therapies is similar, HSCT is the preferred choice of treatment, since it is curative [4]. For most SAA patients lacking a matched sibling donor, IST is the first-line therapy [5].

The initial IST regimen includes a combination of horse antithymocyte immunoglobulin (hATG) and cyclosporine A (CsA), resulting in a hematologic response in 60–75 % of SAA cases [4]. Several studies have reported that children tend to have a higher response rate of 75 % [6–8], compared to older patients (50 %) [9, 10]. Rabbit ATG (rATG) has been used successfully to treat patients with refractory or relapsed SAA following the initial hATG therapy [11, 12]. Recently, some researchers found rATG to be less efficacious than hATG when used as a first-line IST in SAA patients [13–17]. Interestingly, other studies have contradicted these findings by demonstrating similar outcomes in response to both treatment regimens [18–20]. Unfortunately, since hATG is no longer available, rATG is currently the only form of ATG for use in IST in China. On the other hand, both HSCT and IST with ATG remain to be a heavy financial burden for SAA patients even in Western countries. In 2004, porcine antihuman lymphocyte immunoglobulin (pALG) was approved by the State Food and Drug Administration (SFDA) of China as a therapeutic agent. Because of its lower cost (pALG costs only about 40 % of the cost of rATG), it is widely used as a first-line therapy against acquired SAA in China [21]. Bing et al. [22] have shown the excellent outcome resulting from a combined treatment of pALG and CsA in patients with acquired SAA. Despite its rampant usage, thus far, no studies have compared the efficacy of pALG versus rATG when administered with CsA as a first-line treatment against acquired SAA. In this retrospective study, we analyzed the response rates, untoward effects, and treatment-related complications, and overall survival (OS) of 75 SAA patients treated with a single course of either pALG plus CsA or rATG plus CsA. This study would provide a preliminary evidence supporting the use of pALG for treating SAA and vSAA, which could form a basis for future studies to conclusively establish its utility in the clinic.

Patients and methods

Patients

We retrospectively analyzed 75 newly diagnosed SAA patients who were treated with either pALG (n=43) or rATG (n=32) as a first-line therapy between July 2005 and October 2013 in our hospitals. Patients were excluded if they were also diagnosed with dyskeratosis congenita, Fanconi's anemia, or other inherited diseases associated with bone marrow failure. No patients enrolled in this study had a history of viral infection or exposure to drugs or other toxic agents. The exclusion criteria also included the following: patients who were pregnant, who received prior IST, who were diagnosed with other immunological diseases, or tested positive for myelodysplastic syndrome (MDS) based on bone marrow analyses. The baseline peripheral blood cell count for elimination of transfusion and granulocyte colony-stimulating factor (G-CSF) were defined based on the lowest values recorded during the 4 weeks prior to IST. Immediately following diagnosis, most patients were either given no treatment or administered only CsA (3–5 mg/kg/day; 12 in the pALG group and 10 in the rATG group). The two treatment regimens were offered to patients without related human leucocyte antigen (HLA)-matched donors. The final treatment decision was made mainly based on the patients' own preference. All patients provided written informed consent before start of IST.

Diagnosis

In this study, SAA was defined as a condition that satisfied at least two of the following criteria: neutrophil count $<0.5\times10^9$/L, platelet count $<20\times10^9$/L, and reticulocyte count (counted manually) $<20\times10^9$/L [2]. Patients with a neutrophil count $<0.2\times10^9$/L were classified as vSAA [2]. Patients who met the criteria for SAA at the time of diagnosis were classified as SAA-I, while those who progressed from non-severe aplastic anemia (NSAA) to SAA were classified as SAA-II. In the present study, death within the first 60 days following pALG or rATG treatment was defined as early mortality [13].

Porcine Antihuman Lymphocyte Immunoglobulin

SFDA approved pALG (Wuhan Institute of Biological Products, China) (no. S10830001), a polyclonal antibody treated in a manner similar to the ATG obtained from other animals, was used for treatment. Briefly, the immunogen used to generate pALG was human T lymphocytes. Pigs (specific pathogen free) were immunized with human T lymphocytes, and the immunized plasma was treated with human red blood cells (RBCs), placental tissue, and plasma (all blood products were approved by the Chinese Blood Product Standard Committee) to eliminate unwanted and toxic antihuman

antibodies. Polyclonal antibodies were purified from the serum, concentrated, and pasteurized (viral inactivation), by a procedure similar to the preparation of ATGs from other animals [22]. According to the manufacturer's instructions, this type of pALG could be used against the following diseases: SAA, pure red blood cell aplastic anemia, prophylaxis of acute and chronic graft versus host disease following HSCT, and immunosuppression in transplantation procedures for prevention and treatment of graft rejection [22].

Treatment protocol

Patients were first examined for liver function, renal function, and infectious foci, in order to exclude contraindications of therapy. From the start of therapy, all patients were housed in sterile rooms with strict reverse isolation. According to the manufacturers' instructions and Chinese Medical Association's recommendation on dosage [21], the patients were treated with either pALG 30 mg/kg/day or rATG (Thymoglobuline®, Genzyme, Cambridge, MA, USA) 3.75 mg/kg/day from day 1 to day 5. On day 1, a test dose of one tenth of a vial (25 mg pALG or 2.5 mg rATG) diluted in 100 mL of normal saline, was first infused into the subjects for more than 1 h, in order to determine possible adverse reactions to the treatment. In case no severe systemic reaction or anaphylaxis occurred, daily doses of pALG or rATG were then infused intravenously for 12–18 h. Intravenous methylprednisolone at 1–1.5 mg/kg/day and chlorphenamine at 10 mg/day were administered 1 h prior to each daily dose of pALG or rATG. This was followed by infusion of prednisone at 1 mg/kg/day for 30 days, which was gradually tapered to prevent serum sickness. CsA was administered orally at 3–5 mg/kg/day starting on the first day of either pALG or rATG treatment and continued for at least 2 years (the dosage was adjusted to achieve a whole-blood trough level of 200–250 ng/mL for adults and 150–200 ng/mL for children). All patients were subcutaneously injected with G-CSF (5 μg/kg/day) starting from day 8. This dosage was either reduced or terminated when a neutrophil count $\geq 2.0 \times 10^9$/L was reached. Erythropoietin (EPO, 100 IU/kg/day), interleukin-11 (50 μg/kg/day) or recombinant human thrombopoietin (TPO, 300 U/kg/day) were administered when needed. As an adjuvant therapy, all patients were required to ingest testosterone undecanoate capsules (40 mg, bid, for 6–12 months) to stimulate hematopoiesis. RBCs and platelet transfusions were administered in some cases in order to maintain safe blood counts, in compliance with the policies of the local medical center. Prophylactic antibiotics and antifungal and antiviral agents were administered in accordance with institutional guidelines.

Response assessment

Complete response (CR) was defined as transfusion independence associated with age-appropriate hemoglobin count, neutrophil count $>1.5 \times 10^9$/L, and a platelet count $>150 \times 10^9$/L. Partial response (PR) was defined as the inability to any longer meet the criteria for SAA (neutrophil count $>0.5 \times 10^9$/L) as well as a lack of transfusion dependence for platelets or RBCs. Continuous transfusion dependence was classified as no response (NR) [2]. Patients who died within 90 days of therapy and/or underwent HSCT prior to the completion of 6 months following therapy were considered as non-responders [23]. Relapse was defined as a decrease in blood count to values that necessitated IST, transfusions, or HSCT [10].

Safety assessment

During the therapy, toxicity was evaluated for the first 12 weeks, and the complete blood count with differential, liver function, renal function, and comprehensive electrolyte panel were dynamically monitored. Additional examinations were performed where clinical indications were obtained. The Common Terminology Criteria for Adverse Events version 4.0 was used to assess toxicity [24].

Follow-up

The terminal follow-up point was set at January 31, 2014; the median follow-up periods in pALG and rATG groups were 29 months (range=5 to 103 months) and 28 months (range=3 to 100 months), respectively. Follow-up information included a complete blood count (twice a week, before acquired therapy response), liver and kidney functions, bone marrow tests, clinical or laboratory evidence of clonal diseases (including MDS, paroxysmal nocturnal hemoglobinuria (PNH), and leukemia), and cytogenetic analyses. PNH screen was performed annually in all patients.

Statistical analysis

The chi-squared test was used to compare categorical variables; the Student's t test was used to compare continuous variables with normal distribution; and Wilcoxon W test was used to compare continuous variables with non-normal distribution (median values). Survival curves were calculated using Kaplan-Meier analysis and compared using the log-rank test. All statistical analyses were based on the data available on January 31, 2014, and were performed with SPSS version 16.0 (SPSS, Chicago, IL, USA). All P values were two-sided and were considered statistically significant when $P<0.05$.

Results

Characteristics of patients

In the present study, we retrospectively analyzed 75 patients. Among them, 43 (57.33 %) received pALG and 32 (42.67 %) received rATG as first-line therapy. Patient characteristics are shown in Table 1. The two groups were comparable in age, sex, blood cell counts, cytogenetics, presence of PNH clone, and duration of disease. There were no significant differences in absolute neutrophil count (ANC), absolute reticulocyte count (ARC), and platelet count between the pALG and rATG groups. Patients in both groups also received the following drugs: G-CSF (all patients), EPO (34.88 % pALG versus 37.50 % rATG, $P=0.815$), TPO (30.23 % pALG versus 40.63 % rATG, $P=0.350$), IL-11 (51.16 % pALG versus 53.13 % rATG, $P=0.866$). After IST, six patients in the pALG group (13.95 %) and six patients in the rATG group (18.75 %) received allogeneic HSCT ($P=0.575$). The median intervals between IST administration and allogeneic HSCT in the pALG and rATG groups were 11 months (range=8 to 18 months) and 15.25 months (range=10 to 38 months), respectively.

Efficacy of pALG and rATG

After a single course of therapy, there were no significant differences in the overall response rates (OR=CR+PR) at 3, 6, 9, and 12 months after either pALG or rATG treatment (3 months: 41.86 versus 40.62 %, $P=0.914$; 6 months: 66.67 versus 61.29 %, $P=0.635$; 9 months: 69.05 versus 61.29 %, $P=0.490$; 12 months: 69.05 versus 64.51 %, $P=0.684$). The CR rates at 3, 6, 9, and 12 months were similar between the rATG and pALG groups (3 months: 6.25 versus 4.65 %; 6 months: 22.58 versus 19.05 %; 9 months: 25.81 versus 23.81 %; 12 months: 25.81 versus 26.19 %) ($P>0.05$).

We also analyzed the median intervals between treatment and response and found no significant differences between the pALG and rATG groups (105 days (range 20–268) versus 116 days (range 23–277), $P=0.664$). The median times taken for the neutrophil count to reach $\geq 1\times 10^9$/L in pALG and rATG groups were 21 days (range 3–51) and 19 days (range 2–47), respectively ($P=0.832$). The median times required to discontinue the transfusion of RBCs and platelets in pALG and rATG groups were 57 days (range 0–255) and 60 days (range 2–271) for RBCs ($P=0.884$) and 49 days (range 0–270) and 52 days (range 0–295) for platelets ($P=0.842$), respectively. We also monitored the lymphocytotoxicity in response to infusion rATG and pALG and found no significant differences

Table 1 Baseline clinical characteristics of patients in both pALG and rATG groups

Characteristics	pALG	rATG	*P* value
Patients	43	32	
Clinical characteristics			
Median Age (range) (years)	22 (3–64)	23 (3–70)	0.913
Sex (male/female)	29/14	23/9	0.680
Disease status			
SAA	28	21	0.963
vSAA	15	11	0.963
SAA-I	32	22	0.589
SAA-II	11	10	0.589
Blood cell count			
Hemoglobin (g/L, mean±SD)	54.3±7.5	57.6±8.2	0.734
Neutrophil count ($\times 10^9$/L, mean±SD)	0.35±0.04	0.33±0.03	0.617
Platelet count ($\times 10^9$/L, mean±SD)	8.0±1.0	7.0±0.9	0.506
Reticulocyte count ($\times 10^9$/L, mean±SD)	10.4±0.9	11.2±1.1	0.335
Cytogenetic			
Normal karyotype	42	32	1.000
47,XX,+X	1	0	1.000
PNH clone at diagnosis	2	1	1.000
Median interval between diagnosis and treatment (range) (months)	0.9 (0.2–60)	0.75 (0.3–24)	0.817

pALG porcine antihuman lymphocyte immunoglobulin, *rATG* rabbit antithymocyte immunoglobulin, *SAA* severe aplastic anemia, *vSAA* very severe aplastic anemia, *SAA-I* meet the criteria for SAA at the time of diagnosis, *SAA-II* progression from non-severe aplastic anemia to SAA, *PNH* paroxysmal nocturnal hemoglobinuria

in the total lymphocyte counts in a 3-month follow-up between the two groups (first month pALG versus rATG=0.97×10^9/L versus 0.59×10^9/L, P=0.355; second month pALG versus rATG=1.12×10^9/L versus 0.68×10^9/L, P=0.158; third month pALG versus rATG=1.35×10^9/L versus 1.03×10^9/L, P=0.131).

Next, we measured the response rates in the pALG and rATG groups and found them to be equally distributed when patients were classified by sex (P=0.604 and P=1.000, respectively). In addition, although patients <18 years of age tended to display higher OR rates in both groups (pALG 80.00 %, rATG 71.43 %) than patients ≥18 years (pALG 51.85 %, rATG 58.82 %), these differences were not statistically significant (P=0.072 and P=0.724, respectively). We determined the relationship between the OR rates and the interval between diagnosis and treatment and found that, in both pALG and rATG groups, patients with the interval <30 days had significant higher OR rates than those with the interval ≥30 days (83.33 versus 50.00 % and 87.50 versus 40.00 %, P= 0.021 and P= 0.006, respectively). Finally, we analyzed the relationship between OR rates and the neutrophil counts in patients from both groups who received G-CSF and found that, in the pALG group, the OR rate of patients with neutrophil count ≥l.5×10^9/L at day 30 was significantly higher than that in those with neutrophil count <l.5×10^9/L (88.00 versus 47.06 %, P= 0.011). Similar observations were made in the rATG group (83.33 versus 38.46 %, P= 0.028; Table 2).

Interestingly, we found no significant differences in the OR rates between the pALG group and the rATG group in patients <18 years, ≥18 years, SAA, vSAA, SAA-I, SAA-II, an interval between diagnosis and treatment <30 days, ≥30 days, day 30 neutrophil count ≥l.5×10^9/L and <l.5×10^9/L.

Early mortality after IST

One patient with vSAA developed pulmonary infection and died within 1 month of pALG treatment. No early deaths were observed in the rATG group.

Rate of relapse, clonal evolution, and survival following IST

During the follow-up period, one partial responder relapsed 1 year after pALG treatment (after the efficacy evaluation at 12 months) and received HSCT. One complete responder relapsed 2 years after pALG treatment and was subsequently treated through supportive transfusion. In the rATG group, one partial responder relapsed 1 year after treatment (after the efficacy evaluation at 12 months), while one complete responder relapsed 3 years after treatment; both received HSCT after relapse. Two responders at 2 years after treatment and one responder at 3 years after treatment developed a PNH clone in the pALG and rATG groups, respectively. No patient developed any other clonal disease during the follow-up period. We found no statistically significant difference in the relapse rates and clonal evolution rates between the pALG and rATG groups (4.65 versus 6.25 %, P= 1.000, and 4.65 versus 3.13 %, P= 1.000, respectively). In addition, OS in both groups was also comparable at 2 years (pALG 87.4± 6.2 % versus rATG 83.2±7.8 %; P= 0.493) (Fig. 1). We also compared the OS rates at 2 years after treatment between SAA and vSAA patients in each group and found no significant difference between the two types of patients for each treatment regimen (pALG 90.3±6.8 % in SAA versus 81.7±12.3 % in vSAA, P= 0.398; rATG 93.3±6.4 % in SAA versus 64.3± 16.8 % in vSAA, P= 0.060), although the SAA patients

Table 2 Comparison of hematologic responses and clinical data at 12 months after treatment between the pALG and rATG groups

		pALG		rATG	
		Overall response rate (%)	P	Overall response rate (%)	P
Sex	Male	19/29 (65.52)	0.705	14/22 (63.64)	1.000
	Female	10/13 (76.92)		6/9 (66.67)	
Age (years)	<18	12/15 (80.00)	0.072	10/14 (71.43)	0.724
	≥18	14/27 (51.85)		10/17 (58.82)	
AA status	SAA	20/27 (74.07)	0.550	14/20 (70.00)	0.640
	vSAA	9/15 (60.00)		6/11 (54.55)	
	SAA-I	24/31 (77.42)	0.112	16/21 (76.19)	0.117
	SAA-II	5/11 (45.45)		4/10 (40.00)	
Interval from diagnosis to treatment (days)	<30	20/24 (83.33)	0.021	14/16 (87.50)	0.006
	≥30	9/18 (50.00)		6/15 (40.00)	
+30 days neutrophil count(×10^9/L)	>1.5	22/25 (88.00)	0.011	15/18 (83.33)	0.028
	≤1.5	8/17 (47.06)		5/13 (38.46)	

pALG porcine antihuman lymphocyte immunoglobulin, *rATG* rabbit antithymocyte immunoglobulin, *SAA* severe aplastic anemia, *vSAA* very severe aplastic anemia, *SAA-I* meet the criteria for SAA at the time of diagnosis, *SAA-II* progression from non-SAA to SAA

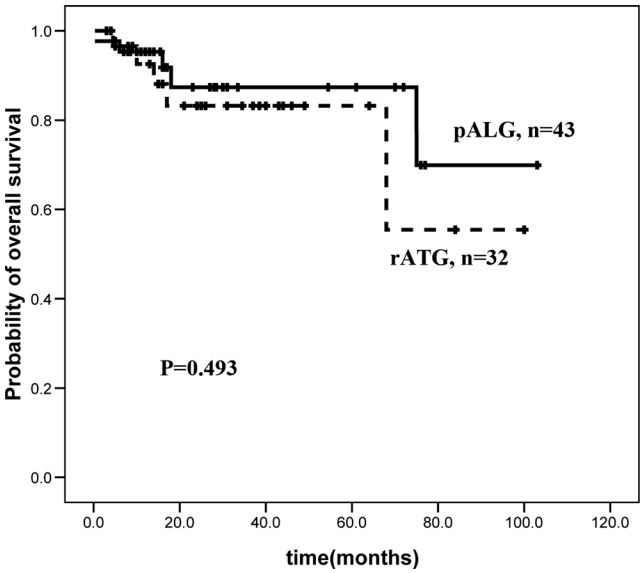

Fig. 1 Comparison of the overall survival (OS) of patients in pALG and rATG groups, analyzed for statistical significance using the log-rank test (P=0.493)

tended to display higher OS rates at 2 years than vSAA patients in both groups.

Toxicity and cause of death after treatment

Acute allergic reactions, including fever, rigor, and skin rash during pALG and rATG therapy were observed in 20.93 % (9/43) and 25.00 % (8/32) patients, respectively (P=0.677), and could be relieved using antiallergic treatment. Serum sickness was also observed in 18.60 % (8/43) and 21.88 % (7/32) patients in pALG and rATG group, respectively (P= 0.726). Next, we examined the effects of IST on liver and kidney functions in both groups. Impaired liver function was seen in 23.26 % (10/43) and 21.88 % (7/32) patients in pALG and rATG group, respectively (P=0.888). The elevated levels of creatinine, indicative of kidney dysfunction, were observed in 6.98 % (3/43) and 6.25 % (2/32) patients in pALG and rATG group, respectively (P=1.000). However, after using reduced glutathione for injection, the liver and kidney functions were restored to normal levels. In the pALG group, infection was observed in 51.16 % (22/43) patients, in addition to a tendency for increased infection rate in vSAA patients compared to SAA patients (66.67 versus 42.86 %). Similarly, in the rATG group, the infection in 62.50 % (20/32) patients, with elevated infection rate being seen in vSAA compared to the SAA patients (72.73 versus 57.14 %). However, these differences were not statistically significant (P= 0.137 and P= 0.631, respectively). We also observed a tendency for increased infection rates in vSAA and SAA patients of the rATG group, compared with the pALG group, but these differences were not statistically significant (P= 1.000 and P= 0.322, respectively). Finally, while comparing the severity of infectious toxicity between pALG and rATG groups, we observed no statistically significant difference in grades 1–2 toxicity (P= 0.509) or grades 3–4 toxicity (P= 0.726) (Table 3).

Finally, we analyzed the differences in the mortality rate between pALG and rATG groups. Four deaths occurred following pALG treatment; one patient died after receiving haploidentical HSCT. Similarly, in the rATG group, four patients died following treatment, while one died after matched sibling HSCT. Infection was identified as the main cause of death in both pALG (4/5 patients) and rATG groups (5/5 patients) (Table 4).

Discussion

Immunosuppressive therapy using pALG has been a popular and affordable treatment for patients with SAA in China. In the present study, we evaluate, for the first time, its efficacy in naive SAA patients, in comparison with rATG. We systematically compared the physiological characteristics of 43 SAA patients treated with pALG and CsA and 32 SAA patients with rATG and CsA as the first IST regimen. Following a single course of pALG, we demonstrated an OR rate of 69.05 % (29/42 patients) and a CR rate was 26.19 % (11/42 patients). In most responders, the therapeutic response occurred within 6 months after treatment. At 3 months, we observed an OR rate of 41.86 % (18/43 patients), consistent with previous findings by Teramura et al. [25]. The response rate and overall survival rate in patients treated with rATG and CsA in our study are better than those reported by groups from the NIH and the EBMT [17, 26], which may be related to the ethnicity and disease status of the patients. G-CSF was administered to all patients, which probably accelerated the short-term recovery of hematopoiesis. The preliminary results indicated that rATG and pALG had similar therapeutic efficacy.

We also found that the response rate was not dependent on the sex of the patient. Unlike previous reports that children had a higher response rate to horse ATG plus CsA than older patients (75 versus 50 %) [6–10], our analysis indicated that, although patients <18 years tended to have higher OR rates in response to both pALG and rATG treatment (80.00 and 71.43 %, respectively) than patients ≥18 years (51.85 and 58.82 %, respectively), these differences were not statistically significant (P=0.072 and P=0.724, respectively). This might be due to the relatively small sample sizes in each group. While some studies have shown higher OR rates in SAA patients than that in vSAA patients following IST [22, 27], others claim no significant differences between the two types of patients [28]. In the present study, we found both SAA and vSAA patients had similar OR rates in response to pALG and

Table 3 Toxicity assessment in both pALG and rATG groups

Toxicity	Grade 1, *n*	Grade 2, *n*	Grade 3, *n*	Grade 4, *n*	Grade 5, *n*	Total, *n*
pALG						
Allergic reaction	4	3	1	1		9
Serum sickness			8			8
Vagina hemorrhage		2				2
Infection						
Upper airway		3	1			4
Pulmonary infections		3	3		1	7
Septicemia			3	1		4
Urinary infection		2				2
Mucositis/stomatitis	2	1				3
Anal infection		2				2
Serum glutamic oxaloacetic transaminase	1	1				2
Serum glutamic pyruvic transaminase	1	1	1			3
Hyperbilirubinemia		1	3	1		5
Creatinine	2	1				3
rATG						
Allergic reaction	3	3	1	1		8
Serum sickness			7			7
Hematuria		1				1
Infection						
Upper airway		3				3
Pulmonary infections		4	3	1	1	9
Septicemia			2	1		3
Urinary infection		1				1
Mucositis/stomatitis	1	1				2
Anal infection		2				2
Serum glutamic oxaloacetic transaminase	1	1				2
Serum glutamic pyruvic transaminase		1	1			2
Hyperbilirubinemia		1	2			3
Creatinine	1	1				2

pALG porcine antihuman lymphocyte immunoglobulin, *rATG* rabbit antithymocyte immunoglobulin

Table 4 Cause of death following pALG and rATG treatments

Cause of death	Time of death (months after pALG/rATG)	Age (years)
pALG (*n*=5)		
Pneumonia	0.5	31
Sepsis	6	28
Sepsis and multiorgan failure	16	39
Cerebral hemorrhage	75	23
Post-HSCT GVHD and pneumonia	18	25
rATG (*n*=5)		
Pneumonia (*n*=3)	4.5/10/17	25/31/31
Post-HSCT pneumonia	14	31
Sepsis	68	23

pALG porcine antihuman lymphocyte immunoglobulin, *rATG* rabbit antithymocyte immunoglobulin, *HSCT* hematopoietic stem cell transplantation, *GVHD* graft versus host disease

rATG at 12 months after treatment. However, patients having an interval of <30 days between diagnosis and treatment showed higher OR rates than those with intervals ≥30 days. It is important to note that four out of five patients in the pALG group and five out of six patients in the rATG group, who experienced an interval of ≥7 months between diagnosis and treatment, were non-responders. This observation emphasizes the importance of the timing of ALG/ATG therapy, which can critically influence the efficacy of treatment. This could be because the longer duration between diagnosis and treatment leads to increased reduction in the number of hematopoietic stem cells, which, in addition to the hematopoietic microenvironment, are affected in aplastic anemia patients. Although the sample size reported here was too small to draw any conclusions, this finding was consistent with previous reports [27]. We believe that it might be prudent to inform patients with prolonged disease duration about possible poor prognosis and if possible, recommend an allogeneic HSCT as the best treatment strategy.

It is known that SAA-II patients generally experience longer duration of disease than SAA-I patients. In the present study, we found that SAA-II patients tended to exhibit lower OR rates following either pALG or rATG treatment than SAA-I patients. However, these differences were not statistically significant, which might be due to the small sample size (especially for small number of patients with SAA-II). Future study with a larger cohort of patients may be able to detect significant differences in OR rates in response to pALG and rATG.

The major adverse effects of pALG treatment were infection (51.16 %), acute allergic reaction (20.93 %) including fever, rigor and skin rash, impaired liver function (23.26 %), and serum sickness (18.60 %). Other undesirable effects included elevated creatinine levels (6.98 %), indicating kidney dysfunction and hemorrhage (4.65 %). Similar adverse effects were observed in both pALG and rATG groups. Most studies on horse or rabbit ATG have shown that vSAA patients incur higher infection rates following treatment [28]. We made similar observations in the present study. We also believe that the administration of CsA might possibly be responsible for impaired liver and kidney functions in these patients. During the follow-up period, we noted similar relapse rates and clonal evolution in the pALG and rATG groups. Taken together, we conclude that the therapeutic efficacy of pALG combined with CsA is not significant different from that of rATG combined with CsA, when administered as a first-line treatment to patients diagnosed with acquired SAA.

Our results suggest that pALG in combination with CsA can be used safely and effectively as a first-line therapy against SAA, with the added advantage of a significantly lower cost, in comparison to hATG or rATG. However, because this report was a retrospective analysis and the sample size of each group was relatively small, our results should be considered preliminary. Further studies with larger cohorts and longer follow-up periods are needed to derive more conclusive findings.

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Conflict of interest The authors declare no conflict of interest.

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·短篇论著·

猪抗人淋巴细胞球蛋白联合环孢素治疗重型再生障碍性贫血患者22例

张兴霞 刘立民 赵广圣 司叶俊 林国强 马爱迪
孙雨梅 张彦明 何广胜 吴德沛

Porcine anti- human lymphocyte immunoglobulin plus cydosporine in the treatment of severe aplastic anemia

Zhang Xingxia, Liu Limin, Zhao Guangsheng, Si Yejun, Lin Guoqiang, Ma Aidi, Sun Yumei, Zhang Yanming, He Guangsheng, Wu Depei

Corresponding author: Zhang Yanming, Department of Hematology, Second People Hospital of Hua'ian City, Huai'an 223002, China. Email:zhangyanming2005@126.com

重型再生障碍性贫血(SAA)是一种获得性骨髓造血功能衰竭综合征,许多患者在短期内死于严重感染和出血[1-2]。抗人胸腺细胞球蛋白(ATG)联合环孢素(CsA)的免疫抑制治疗(IST)有效率可达60%~80%,已成为年龄≥40岁或年龄<40岁但无HLA相合同胞供者SAA患者的首选治疗方案[3-5]。但是目前猪抗人淋巴细胞球蛋白(p-ALG)治疗SAA相关报道少见,我们总结我院p-ALG联合CsA治疗SAA患者的疗效、并发症及不良反应,现报道如下。

病例和方法

1. 病例:2005年7月至2013年3月应用p-ALG联合CsA治疗SAA患者22例,男14例,女8例,中位年龄23(3~64)岁,其中儿童患者(年龄≤14岁)9例,成人患者(年龄>14岁)13例。诊断标准参照文献[6]。患者初诊未治时中性粒细胞绝对计数(ANC)中位数为0.37(0.04~0.90)×10⁹/L、HGB中位数为55(29~90)g/L、PLT中位数为8(2~20)×10⁹/L、网织红细胞计数(Ret)中位数为10.9(0~19.1)×10⁹/L。发病到p-ALG治疗的中位时间为15(5~4 768)d,19例患者在p-ALG治疗前未接受任何治疗,3例在p-ALG治疗前接受CsA(3~5 mg·kg⁻¹·d⁻¹)、雄激素及对症支持等治疗。

2. 治疗方案:p-ALG(武汉生物制品研究所产品)30 mg·kg⁻¹·d⁻¹,静脉滴注12~18 h,连续使用5 d。第1天先以p-ALG 25 mg加至100 ml生理盐水中静脉缓慢滴注1 h,如无不良反应,则当日余量溶于500 ml生理盐水中,匀速静脉滴注维持。成人患者用药前30 min口服异丙嗪25 mg及静脉注射甲泼尼龙40 mg(第1~5天),应用地塞米松10 mg/d静脉滴注同步维持以预防过敏反应;儿童患者用药前30 min口服异丙嗪0.25~0.50 mg/kg及静脉注射甲泼尼龙1.5 mg·kg⁻¹·d⁻¹(第1~5天),应用地塞米松0.15 mg·kg⁻¹·d⁻¹静脉滴注同步维持。p-ALG治疗结束后1周开始地塞米松逐渐减量,4周后停药,根据有无血清病调整用量。予CsA 3~5 mg·kg⁻¹·d⁻¹,口服,根据患者血药浓度肝肾功能调整用量,维持治疗2年以上,逐步减量。所有患者IST期间,均入住无菌层流病房。

3. 疗效评价:疗效评定采用英国血液学标准委员会标准[6],①完全治疗反应(CR):HGB达同龄人正常水平,PLT>150×10⁹/L,ANC>1.5×10⁹/L。②部分治疗反应(PR):脱离输注血制品,血液学检查好转,不再符合SAA标准。③无治疗反应(NR):患者未脱离血制品输注支持治疗,血液学检查仍符合SAA标准。CR、PR均为治疗有效。复发定义为获得治疗反应患者疗效维持时间至少达3个月,血常规参数再下降,需要进一步IST或依赖血制品输注。如患者仅出现短暂血常规指标轻度下降,不视为复发。限定1年内获得治疗反应为治疗有效,治疗12个月后才开始血液学改善者仍视为IST失败,不计入治疗有效。

4. 随访:中位随访时间为24(6~98)个月。在获得治疗反应前,患者每周至少进行2次血常规检测,并分别于IST后3、6、9、12个月进行骨髓细胞形态学、细胞遗传学检查及外周血细胞锚链蛋白检测以了解有无晚期克隆性并发症。

结 果

1. 疗效:22例患者中,CR 14例(63.6%)、PR 4例(18.2%),NR 4例,总有效率81.8%(22例中18例)。治疗后不同时间点疗效见表1。治疗有效患者起效的中位时间为90(23~277)d,ANC≥1×10⁹/L的中位时间为14(2~47)d,脱离红细胞输注的中位时间为38(0~255)d,脱离血小板输注的中位时间为27(0~270)d。CR患者中1例2年后复发;1例2年后检测到阵发性睡眠性血红蛋白尿(PNH)克隆,随访期间未有溶血表现。4例NR患者中3例IST后1年半行异基因造血干细胞移植后治愈,1例在治疗后9个月更换使用兔ATG治疗后仍无效。无一例进展为其他克隆性疾病。

2. 疗效分析:14例男性患者中11例有效,8例女性患者中7例有效;9例儿童患者中8例有效,13例成人患者中10

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作者单位:223002 江苏淮安,徐州医学院附属淮安医院、淮安市第二人民医院血液科(张兴霞、刘立民、赵广圣、司叶俊、林国强、马爱迪、孙雨梅、张彦明);苏州大学附属第一医院血液科(何广胜、吴德沛)

通信作者:张彦明,Email:zhangyanming2005@126.com

表1 猪抗人淋巴细胞球蛋白联合环孢素治疗22例重型再生障碍性贫血患者不同时间点的疗效(例)

治疗时间	CR	PR	NR	有效率(%)
3个月	3	12	7	68.2
6个月	11	6	5	77.3
9个月	13	5	4	81.8
12个月	14	4	4	81.8

注:CR:完全治疗反应;PR:部分治疗反应;NR:无治疗反应

例有效;13例发病至p-ALG治疗时间间隔≤30 d患者有效,仅有4例发病至p-ALG治疗时间间隔超过30 d者有效;仅有1例在应用p-ALG后第30天(+30 d)ANC > 1.5×10^9/L者无效,而+30 d ANC≤1.5×10^9/L的8例患者中仅有4例有效。

3. 不良反应:p-ALG治疗过程中2例患者出现发热、寒战、皮疹、水肿、关节痛、血清病等,均较轻微,经适当处理后有效控制。3例出现肝肾功能损伤及齿龈增生,考虑与CsA联合使用有关,暂停CsA或调整剂量后恢复正常。22例患者中,6例在p-ALG治疗后3个月内发生感染,其中上呼吸道感染2例、肺部感染2例、败血症1例、口腔黏膜感染1例,均经采用积极的抗感染和支持治疗后得以控制,未发生感染性休克和弥散性血管内凝血(DIC)等危象,未出现治疗相关死亡。

讨 论

ALG/ATG联合CsA适用于40岁以上的SAA或极重型再生障碍性贫血(VSAA)患者及40岁以下无合适同胞供者的SAA或VSAA患者。本研究中我们应用p-ALG联合CsA治疗22例SAA患者总体有效率为81.8%,与国内韩冰等[7]报道的p-ALG治疗SAA患者的有效率(82.2%)及曹慧敏等[8]报道的兔ATG治疗成人SAA患者的有效率(83.3%)相当,但高于国外报道的马或兔ATG治疗SAA患者的有效率[2,9],进口马或兔ATG的治疗费用相对昂贵,相比之下国产p-ALG可以明显减轻患者的经济负担。

本研究结果显示,IST治疗后3个月3例患者达到CR,治疗效果好,该3例患者在诊断时均经详细检查,排除急性造血功能停滞及其他因素所致的全血细胞减少,治疗后回顾性诊断仍考虑为SAA。Scheinberg等[10]认为SAA患者发病年龄低者预后好,但Ahn等[11]认为ATG治疗SAA预后与患者年龄无关。本组22例患者的中位年龄为23(3~64)岁,40岁以上的患者仅2例,儿童患者9例,整体相对较年轻。此外,4例病程较长患者中3例自发病至应用p-ALG间隔分别为9个月、5年、13年的患者无一例有效,其余18例患者病程均≤3个月,IST治疗后6个月14例有效,提示病程早期IST效果好。国外也有类似的报道[12]。推测SAA患病时间长可使体内残存干细胞过少,早期应用ATG/ALG可及时抑制T细胞及骨髓抑制因子对造血干细胞和(或)造血微环境的损伤,遏制病情进展,阻止红髓被黄髓取代,促进造血功能恢复。

Tichelli等[1]报道对于IST后接受G-CSF患者,若在+30 d时中性粒细胞仍缺乏反应,预示对ALG/CsA反应差;对于G-CSF治疗有效患者,推测可能的机制为患者骨髓储备造血功能较好,抑制异常免疫后其造血功能得以恢复。

虽然本组患者随访时间6~98个月长短不一,未能评价长期生存和晚期克隆性血液学异常情况,但我们认为这并不影响对p-ALG疗效进行评价。复发主要由CsA过早停药和过快减量导致。本研究中1例患者治疗后复发,考虑可能与CsA药物减量快有关,重新加用CsA治疗仍有效。IST后克隆性血液学异常包括出现PNH克隆及向骨髓增生异常综合征/急性髓系白血病(MDS/AML)转化。本组1例患者2年后检测到PNH克隆。目前尚不清楚出现克隆性血液学异常是SAA自然病程还是与IST相关。Kumar等[13]研究发现造血生长因子(如G-CSF)与ATG联用会增加 MDS/AML的发生的危险;但Locasciulli等[14]在对114例SAA患者IST联合G-CSF与IST的对照研究中发现,G-CSF剂量达36 000 μg/例、使用时间超过4个月并不会增加患者发生克隆性疾病的危险。关于SAA患者是否应该使用 G-CSF治疗、G-CSF的使用剂量及治疗时间尚未达成共识。

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聚乙二醇化重组人G-CSF在淋巴瘤化疗中预防性应用的回顾性分析

张群岭 刘晓健 沈维娜 王佳琛 洪小南 郭晔

The retrospective analysis of prophylactic administration of pegylated recombinant human granulocyte colony stimulating factor in lymphoma patients undergoing chemotherapy *Zhang Qunling, Liu Xiaojian, Shen Weina, Wang Jiachen, Hong Xiaonan, Guo Ye*

Corresponding author: Guo Ye, Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai 200032, China. Email:pattrickguo@gmail.com

骨髓抑制是淋巴瘤患者接受化疗后的常见不良反应,主要表现为中性粒细胞缺乏(粒缺)和发热性粒缺(febrile neutropenia,FN)。Ⅳ度粒缺或FN往往是后续化疗的减量依据。作为对化疗高度敏感的肿瘤,淋巴瘤化疗方案的剂量强度与疗效密切相关[1]。由于淋巴瘤患者本身具有一定的免疫缺陷以及治疗方案中包含多个免疫抑制药物,FN导致的感染往往成为化疗相关性死亡的首要原因。

为了避免降低化疗剂量和减少FN的发生,通常的做法是在化疗后初级或次级预防性应用G-CSF[2]。关于初级预防的指征,多个指南推荐常规用于FN风险超过20%的化疗方案。对于风险介于10%和20%的方案,欧洲癌症研究与治疗组织(EORTC)的指南建议综合评价FN的其他高危因素如高龄(≥65岁)、贫血、骨髓造血功能或营养状态差等进行个体化的治疗决策[3]。近期国内上市的聚乙二醇修饰的G-CSF(PEG-rhG-CSF),半衰期长达数十小时,只需要在化疗结束后进行1次注射,应用便利[4-5]。我们回顾性分析43例淋巴瘤患者预防应用PEG-rhG-CSF病例资料,现报道如下。

病例和方法

1. 病例:2013年3月至2013年12月在我院接受化疗并预防性使用PEG-rhG-CSF的淋巴瘤患者43例,淋巴瘤诊断参照2008年的WHO标准[6]。其中17例接受了初级预防,26例接受了次级预防,所有患者均纳入不良反应分析。接受初级预防的17例患者中,1例82岁的患者因接受减量的mini-CHOP方案(环磷酰胺、长春新碱、多柔比星和泼尼松)而剔除。9例接受高强度化疗方案(FN风险超过20%),5例高龄(≥65岁),2例治疗前WBC < 4×10^9/L。接受次级预防的26例患者均在前期化疗出现Ⅳ度粒缺或FN,其中3例患者因在接受预防治疗的同时化疗剂量下调而剔除。在纳入最终疗效分析的2组共39例患者中,34例已经完成了化疗,5例仍在治疗过程中。

2. 病理类型和化疗方案:入组的淋巴瘤病理类型包括弥漫大B细胞淋巴瘤、滤泡性淋巴瘤、套细胞淋巴瘤、小淋巴细胞性淋巴瘤、NK/T细胞淋巴瘤和血管免疫母T细胞淋巴瘤。化疗方案包括CHOP(环磷酰胺、长春新碱、多柔比星、泼尼松)、R-CHOP(利妥昔单抗、环磷酰胺、长春新碱、多柔比星、泼尼松)、G-CHOP(obinutuzumab、环磷酰胺、长春新碱、多柔比星、泼尼松)、R-DHAP(利妥昔单抗、地塞米松、阿糖胞苷、顺铂)、O-DHAP(ofatumumab、地塞米松、阿糖胞苷、

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作者单位:200032 上海,复旦大学附属肿瘤医院肿瘤内科

通信作者:郭晔,Email:pattrickguo@gmail.com

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ORIGINAL ARTICLE

Porcine antilymphocyte globulin (p-ALG) plus cyclosporine A (CsA) treatment in acquired severe aplastic anemia: a retrospective multicenter analysis

Jia Wei · Zhiping Huang · Jingming Guo · Youshan Zhang · Chunyan Wang · Xianmin Zhu · Yicheng Zhang

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Abstract Severe aplastic anemia (SAA) is a life-threatening bone marrow failure syndrome characterized by pancytopenia and hypocellular bone marrow. Antithymocyte globulin (ATG) is the drug of choice for immunosuppressive therapy (IST) in patients with SAA ineligible for allogeneic stem cell transplantation. This study aimed to evaluate the efficacy and safety profile of porcine antilymphocyte globulin (p-ALG) plus CsA in the treatment of acquired SAA. Clinical information of 69 SAA patients treated with p-ALG plus CsA was collected and retrospectively analyzed for early mortality, response rate, survival rate, side effects, and other complications. The median age at diagnosis was 27 years (range 14 to 52). The overall response rate was 76.8 % with a 90-day median response time (range 30~360 days). Overall response rates at 3, 6, 12, 18, and 24 months were 63.8, 73.9, 76.8, 75.4, and 75.4 %, respectively. The median follow-up time for surviving patients was 24 months (range 4~44 months) and the 2-year overall survival (OS) rate was 88.4 %. The disease-free survival (DFS) rate at 2 years was 85.5 %. Older age (≥45 years), very (v)SAA subgroup, and lower baseline absolute lymphocyte count ($<1\times10^9$/L) were independent unfavorable predictors of overall survival ($p<0.05$). Less than one third of patients had serum sickness or allergic reaction during ALG therapy, but symptoms could easily be relieved by steroid treatment; 27.54 % had mild hepatic impairment. Taken together, p-ALG showed similar efficacy and safety profiles to rabbit or horse ATG in IST of acquired SAA. It can be a suitable alternative preparation for rabbit ATG with the great advantage of lower medical expenses.

Keywords Severe aplastic anemia · Porcine ALG · Overall response · Overall survival

Introduction

Severe aplastic anemia (SAA) is a serious condition caused by immune-triggered bone marrow failure. Delayed treatment is associated with high mortality [1]. Allogeneic bone marrow transplantation and immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine A (CsA) have dramatically changed the course of this illness, with the 10-year survival rate for either treatment now being about 70 % [2]. IST with ATG plus cyclosporine A has become the standard treatment for patients with SAA ineligible for allogeneic stem cell transplantation [3]. In western countries, two ATG preparations for IST are currently available: the historically first used preparation from horse and the later approved preparation from rabbit. The response rate for IST containing these two ATG preparations ranged from 60 to

Jia Wei and Zhiping Huang contributed equally to this work.

J. Wei · C. Wang · X. Zhu · Y. Zhang (✉)
Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jie-Fang Avenue, 430030 Wuhan, China
e-mail: yczhang@tjh.tjmu.edu.cn

Z. Huang
Department of Hematology, Jingzhou Central Hospital, 434020 Jingzhou, China

J. Guo
Department of Hematology, Yichang Central People's Hospital, China Three Gorges University, 443003 Yichang, China

Y. Zhang
Department of Hematology, the First People's Hospital of Jingzhou, Yangtze University, 434000 Jingzhou, China

80 % [4–6]. In recent randomized or prospective studies, it was found that rabbit ATG was inferior to horse ATG as a first-line treatment for severe aplastic anemia [6–8]. Horse ATG, however, is not available in China and most clinical centers use rabbit (r)-ATG with cyclosporine A as first-line treatment in SAA for patients not eligible for bone marrow transplantation. The response rate for this was reported between 75 and 85 % [9–11]. Although porcine antilymphocyte globulin (p-ALG) was approved in China to treat SAA in 2004, systematic investigation of this preparation in the treatment of SAA was seldom reported. Compared to horse or rabbit ATG, p-ALG is substantially cheaper, lowering the costs of therapy by 30 to 50 % per cycle of treatment ($443 vs $201/bottle). In this study, we have performed a multicenter retrospective analysis of p-ALG plus CsA in the treatment of acquired SAA.

Methods

Patients

Sixty-nine consecutive SAA patients who had no suitable related donor for allogeneic stem cell transplantation or were over 45 years old received IST from 2009 to 2013. The medical records of these patients who underwent IST were retrospectively reviewed in detail. The IST protocols described in this study were approved by the ethics committee of their own hospitals. Appropriate informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

All diagnoses were reviewed by at least three senior hematologists independently. Fanconi anemia, paroxysmal nocturnal hemoglobinuria (PNH), and other constitutive bone marrow failure syndromes were excluded before IST. Chromosomes were assayed after in vitro exposure of lymphocytes to diepoxybutane to exclude Fanconi anemia. In addition, all patients received Fanconi anemia mutation analysis including 43 exons of FANCA, 14 exons of FANCC, and 14 exons of FANCG by PCR and Sanger sequencing. The cytogenetic karyotypes, MDS FISH panel (−5/5q−, −7/7q−, +8, 20q−), bone marrow biopsy and immunohistology (including CD34, CD31, CD42, CD61, CD3, CD15, CD20, and others) were done prior to the ALG treatment.

PNH clone was detected with flow cytometric analysis (BD Bioscience, San Jose, USA) of CD55 and CD59 expression (BD Immunocytometry Systems, San Jose, CA) on red blood cells and glycosyl-phosphatidylinositol expression on neutrophils. The cutoff value used to characterize the PNH clone was 3.0 % in erythrocyte lineage and 0.1 % in granulocyte lineage [12, 13]. No baseline PNH clone was found by flow cytometry in our series. The diagnosis of SAA required bone marrow cellularity less than 25 % and at least two of the following three criteria from peripheral blood counts: platelet counts $\leq 20\times10^9$/L, neutrophil count $\leq 0.5\times10^9$/L, and corrected reticulocyte count ≤1 %. Very severe aplastic anemia was defined as meeting the SAA criteria and having neutrophil counts less than 0.2×10^9/L [14].

Immunosuppressive regimens

All patients underwent skin test to assess allergic hypersensitivity. p-ALG® (Wuhan Institute of Biological Products, China, State Medical Permit No. S10830001) was approved by the State Food and Drug Administration (SFDA) in 2004. All patients were treated with p-ALG intravenously 25 mg/kg/day followed by 1.5 mg/kg/day methylprednisolone on days 1–5 and then 1 mg/kg/day prednisolone for 30 days and tapered gradually to prevent serum sickness. CsA 3–5 mg/kg/day was used for at least 2 years. The CsA dose was adjusted to achieve a whole-blood trough level of 200 ng/mL. Complete blood count as well as biochemistry parameters was monitored accordingly. Granulocyte colony-stimulating factor (G-CSF) was administered for all patients at 5 μg/kg/day subcutaneously everyday until day 30 and then administered if neutropenia occurred, and the dose was adjusted with neutrophil counts. As prophylaxis against *Pneumocystis carinii* pneumonia, all patients received TMP-SMX during the treatment period. Oral acyclovir and voriconazole were given for viral and fungal prophylaxis.

Response criteria

Complete response was defined as independence from transfusion, associated with normal hemogram parameters. Partial response was defined as no longer meeting the criteria for SAA without normalization of blood counts and no transfusion dependence for platelet and/or red blood cells. Continuous transfusion dependency was classified as no response. Relapse was defined as a decrease in blood counts to values either requiring transfusions or needing reinstitution of IST or HSCT [15]. Clonal evolution was defined as newly diagnosed clonal cytogenetic abnormalities or characteristic dysplastic or leukemic changes in the bone marrow. Early mortality was defined as death within the first 60 days after ATG treatment [16].

Follow-up

Overall survival (OS) was measured from the date of IST to either death from any cause or the date of last follow-up through June 30, 2014. Disease-free survival (DFS) was calculated from the date of IST to the first day of either recurrence or clonal transformation or death. Follow-up information consisted of a complete blood count, blood biochemistry, the assessment of bone marrow changes, and clinical or molecular evidence of clonal disease.

Statistical methods

Software SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was applied to perform all statistical analyses. The overall survival and disease-free survival was analyzed by the Kaplan-Meier method. Log rank test and chi-square test were applied to calculate the significance of differences between risk groups with a level of significance of $p<0.05$. Cox regression was used to assess independent prognostic predictors in multivariate analysis with a stepwise selection algorithm of important covariates.

Results

Patients' characteristics

A total of 69 SAA patients received p-ALG plus CsA as IST protocol. The male/female ratio was 1.56. Median age at diagnosis was 27 years (range 14 to 52). Median time from diagnosis to IST was 18 days (range 4–583 days). At diagnosis, median neutrophil count was 0.32×10^9/L (range $0\sim2.87\times10^9$/L); median hemoglobin count was 62 g/L (range 31.4~140 g/L); median platelet count was 9×10^9/L (range $1\sim41\times10^9$/L). Of the patients, 89.9 % were classified as idiopathic SAA; 7.2 % were hepatitis-related SAA and 2.9 % drug-induced SAA. All patients received only one course of p-ALG treatment. The patients' characteristics are shown in Table 1.

Table 1 Clinical characteristics of the 69 patients

Patient characteristics	No. of patients (%)
Male/female	1.56
Median age at diagnosis (range)	27 years (14–52)
Cause of aplastic anemia	
Idiopathic	62 (89.9 %)
Hepatitis-related	5 (7.2 %)
Drug-induced	2 (2.9 %)
Median time from diagnosis to IST (range)	18 days (4–583 days)
Response to IST	
Complete response	39 (56.5 %)
Partial response	14 (20.3 %)
No response	16 (23.2 %)
Overall response at 3 months*	44 (63.8 %)
Overall response at 6 months*	51 (73.9 %)
Overall response at 12 months*	53 (76.8 %)
Overall response at 18 months*	52 (75.4 %)
Overall response at 24 months*	52 (75.4 %)
Median time of follow-up (range)	24 months (4–44 months)
Median neutrophil count, 10^9/L (range)	0.32 (0–2.87)
Median hemoglobin, g/L (range)	62 (31.4–140)
Median platelet count, 10^9/L (range)	9 (1–41)
Median reticulocyte count, 10^9/L (range)	9.7 (2.4–82.6)

*Overall response= Complete response + Partial response

Early death and overall response

Of the 69 patients, 7 (10.14 %) died within 60 days. The highest early death rates in single center was 33.3 % (4/12), followed by 13.04 % (3/23). Six of them died of severe infection (four died of severe sepsis and two died of invasive fungal infection) and all six had preexisting infections. One died of cranial hemorrhage (Table 4). Fifty-three patients (76.8 %) became transfusion independent after one course of p-ALG with 56.5 % complete response and 20.3 % partial response. The median response time was 90 days (range 30~360 days). The overall response rates at 3, 6, 12, 18, and 24 months were 63.8, 73.9, 76.8, 75.4, and 75.4 %, respectively. The median neutrophil counts ($\times10^9$/L) at 3, 6, 12, 18, and 24 months were 1.89 (0.1~14.5), 2.01 (0.51~13.19), 2.35 (0.54~4.13), 2.09 (0.48~3.25), and 2.07 (0.47~3.25), respectively. The median hemoglobulin levels (g/L) at 3, 6, 12, 18, and 24 months were 105 (37~149), 122 (31~158), 138 (22~164), 140 (39~156), and 121 (32~149), respectively. The median platelet counts ($\times10^9$/L) at 3, 6, 12, 18, and 24 months were 52 (1.4~213), 125 (1~251), 201 (2~324), 198 (2~236), and 215 (7~273), respectively. No prognostic factors were correlated with the overall response rate in our study (Table 2).

Overall survival and relapse

The median follow-up time for surviving patients was 24 months (range 4~52 months) and the 2-year OS rate was 88.4 % (Fig. 1a). The estimated DFS rate at 2 years was 85.5 % (Fig. 1b). In univariate analysis of prognostic factors for overall survival, older age (≥45 years), very (v)SAA subgroup, preexisting infection, and lower absolute lymphocyte count ($<1\times10^9$/L) predicted inferior survival rate ($p<0.05$). In multivariate Cox regression analysis, older age (≥45 years), vSAA subgroup, and lower baseline absolute lymphocyte count (ALC) ($<1\times10^9$/L) were independent predictors of OS rate ($p<0.05$, Fig. 2 and Table 3). One patient showed clonal evolution (1.4 %) and transformed to myelodysplastic syndrome (RAEB-I) during the follow-up period. Only one patient relapsed (from partial response to transfusion dependent) after 23 months (Table 4).

Kinetics of lymphocyte depletion after porcine ALG

The kinetic lymphocyte depletion after porcine ALG was depicted in Fig. 3. During the first week after ALG, the lymphopenia was profound. The lymphocytes gradually recovered after 2 weeks of ALG treatment.

Table 2 The univariate analysis of prognostic factors for overall survival and overall response

Factors	Subtype	Number (percentage)	Mean OS (months)	*p* value[a]	Overall response (%)	Nonresponse (%)	*p* value[b]
Sex	Male	42 (60.9)	46.3	0.955	83.3	16.7	0.129
	Female	27 (39.1)	36.1		66.7	33.3	
Age	<45 years old	63 (91.3)	47.26	**0.048**	77.8	22.2	0.157
	≥45 years old	6 (8.7)	27.39		66.7	33.3	
SAA subtypes	vSAA	17 (24.6)	33.82	**0.013**	58.8	41.2	0.090
	SAA	52 (75.4)	49.04		82.7	17.3	
Time intervals	<60 days	58 (84.1)	46.77	0.463	81	19	0.129
	≥60 days	11 (15.9)	28.61		54.5	45.5	
Preexisting infection	None	48 (69.6)	43.27	**0.035**	82.0	18.0	0.181
	Existed	21 (30.4)	40.06		63.2	36.8	
Baseline ALC	$\geq 1\times10^9$/L	36 (52.2)	50.61	**0.019**	86.1	13.9	>0.05
	$<1\times10^9$/L	33 (47.8)	36.78		66.7	33.3	
Baseline ARC	$\geq 25\times10^9$/L	16 (23.2)	–	0.11	75.5	24.5	>0.05
	$<25\times10^9$/L	53 (76.8)	–		81.3	18.7	

Significance of values in bold is $p<0.05$

Time intervals was calculated from time of diagnosis to time of IST; – no statistics are computed since in the group absolute reticulocyte at diagnosis greater than 25×10^9/L, all cases are censored

ALC absolute lymphocyte count, *ARC* absolute reticulocyte count

[a] *p* value was calculated by the Kaplan-Meier method

[b] *p* value was calculated by chi-square test

Side effects

Of the total 69 patients, 15 patients (21.73 %) had serum sickness that could be relieved by steroid treatment. Twenty-three patients (33.33 %) had allergic reaction (including fever and skin rash) during ALG therapy that could be relieved by antiallergic treatment. Nineteen patients (27.54 %) had mild elevated liver enzymes. No elevated serum creatinine levels, fatal serum sickness, or anaphylactic shock was reported in this cohort.

Discussion

IST with ATG and CsA is an effective front-line treatment for SAA when there is no histocompatible sibling donor or the patient's age is generally over 45 years old. In western countries, standard initial IST is horse ATG plus CsA, which induces hematologic recovery in 60 to 70 % of cases and excellent long-term survival among responders, as shown in several large prospective studies in the USA, Europe, and Japan [17–20]. A more lymphocytotoxic agent, rabbit ATG, was

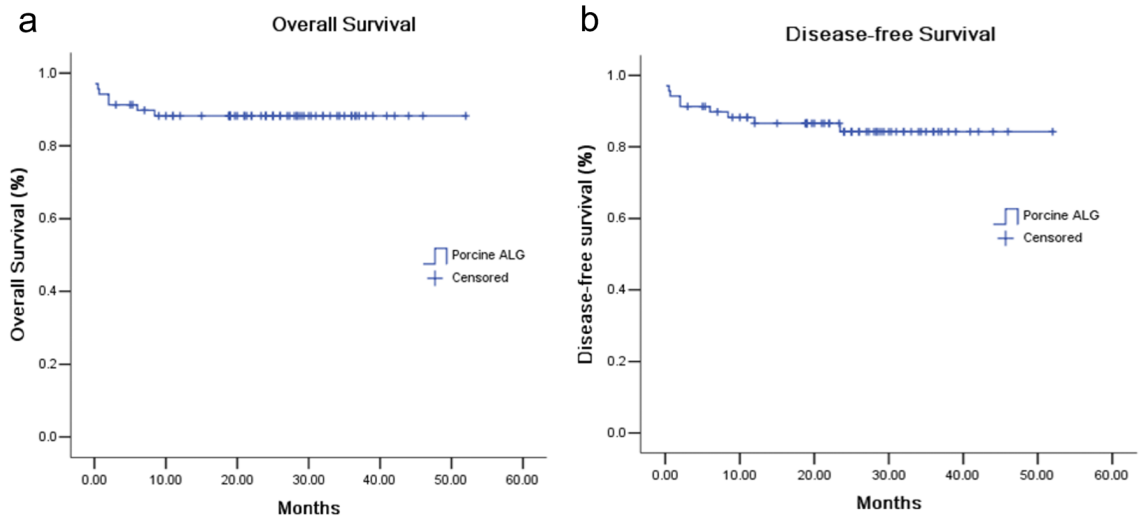

Fig. 1 Kaplan-Meier curve of overall survival (OS) and disease-free survival (DFS) of 69 patients. The 2-year OS rate was 89.86 %; the estimated survival time was 46.833±1.851 months (**a**). The DFS rate at 2 years was 85.5 % (**b**)

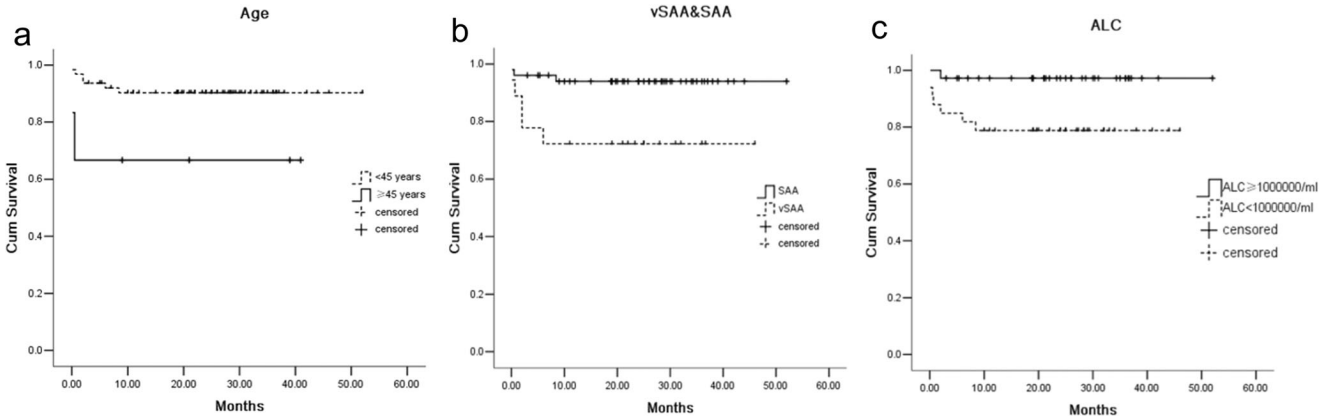

Fig. 2 Comparison of overall survival in relation to age (**a**), SAA subgroup (**b**), and ALC count (**c**). The older age (≥45 years), vSAA subgroup, and low ALC count ($<1\times10^9$/L) had a significantly shorter OS rate compared to the SAA group ($p<0.05$)

successful in salvaging patients with refractory or relapsed SAA after initial treatment with horse ATG [21, 22]. Whether horse (h)-ATG was still superior to r-ATG was controversially discussed [6, 23–27]. In China, rabbit ATG was the only available agent before 2004, but most patients could not afford this expensive drug which is not covered by any insurances. In recent years, some centers in China tried p-ALG to substitute r-ATG in IST. Bing et al. used p-ALG and results showed 83.3 % overall response with a 90-day median response time and 87.5 % 1.5 years survival rate [28]. Despite biological similarities in manufacturing, there may exist many differences in pharmacokinetics and effects on neutrophils, lymphocyte subsets, and cytokine release between the three types of ATG [29]. Any one of these differences might be related to their clinical efficacy in suppressing the immune system and restoring hematopoiesis in SAA patients. Table 5 has summarized the clinical efficacy of IST with different ATG preparations in recent published data. Our retrospective data shows that the response rate and 2-year OS rate are not inferior to the results reported with rabbit ATG in China [9–11] and horse/rabbit ATG in western countries [6, 15–18, 20].

In this study, 7 of 69 patients died within 60 days. Patients who had preexisting infections were more likely to develop fatal infection during or after IST. Platelet refractoriness was the cause of fatal cranial hemorrhage in another patient. The above results indicate that, irrespective of the stage of IST, infections are the most common cause of death. More than one third of patients treated in one center have encountered early death which is obviously higher than other centers. This suggests that IST should be performed in centers with expertise in managing it to avoid early death. According to our study, no serious side effects were observed. Common side effects including skin rash, serum sickness, and mildly elevated liver enzymes were also observed in studies investigating horse or rabbit ATG.

In our series, the relapse rate was lower than other published data in western countries but quite similar with other Chinese patients [9–11, 28, 30] irrespective of the type of ATG. There may have two reasons for the lower relapse rate. First, all patients in our cohort received G-CSF support. G-CSF was previously proven that it had some benefits in terms of the promotion of hematopoietic recovery and suppression of relapse rate [31], but further follow-up is required to elucidate whether it increases the risk of clonal evolution. Second,

Table 3 The multivariate analysis of prognostic factors of OS

Multivariate variables	Odds ratio	95 % CI	*p* value[a]
Age (≥45/<45 years old)	5.268	1.352–45.849	0.022
SAA subtype (vSAA/SAA)	5.720	1.385–26.585	0.017
Baseline ALC (≥1/<1×109/L)	4.879	1.318–102.285	0.027

ALC absolute lymphocyte count

[a] Forward and backward Wald test

Table 4 Information of early death and other late events

	Number (percentage)	Cause
Early death	7 (10.14)	Infection (85.7 %); hemorrhage (14.3 %)
Late death	1 (1.4)	Infection (100 %)
Clonal evolution	1 (1.4)	MDS (RAEB-I) after 3 months of IST
Relapse	1 (1.4)	Become transfusion dependent after 23 months of IST
Late infection events	5 (7.2)	Invasive fungal infection (60 %); bacteremia (40 %)

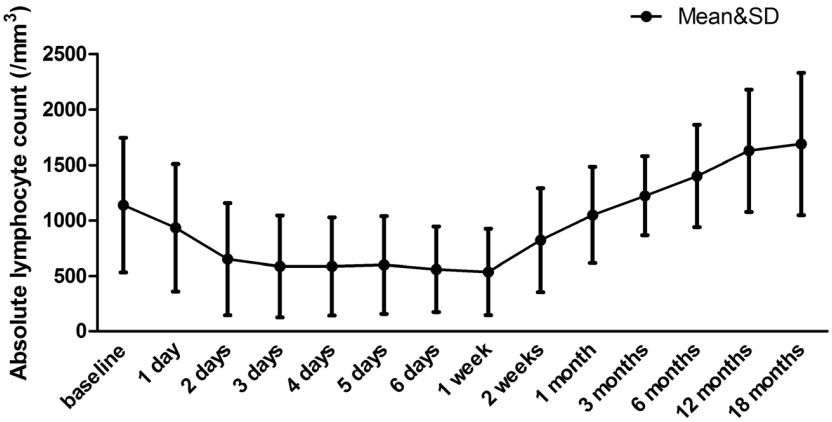

Fig. 3 Kinetics of absolute lymphocyte counts after p-ALG treatment

most patients (95.7 %) are still receiving CsA at the time of analysis. It has been also demonstrated that the combination of ATG and CsA is superior to ATG or CsA alone in terms of hematologic response [18, 32]. This may be another reason for the low relapse rate. Although clonal evolution may occur in approximately 10 to 15 % of patients [24], some clonal abnormalities appear to be transient and may be omitted. In addition, most clonal evolution occurred as the late event of SAA. More clonal evolution may be observed if the follow-up time prolonged.

Although our study was limited by its retrospective nature and the short time of follow-up, the 88.4 % 2-year OS rate showed excellent efficacy of p-ALG. Thus, p-ALG may serve as a potential alternative for r-ATG. In this study, vSAA subgroup, older age, and low ALC count were three independent unfavorable predictors of overall survival. A higher baseline absolute reticulocyte count (ARC) and ALC may indicate better residual marrow function and the presence of a sufficient number of stem cells to sustain blood cell production after IST [33]. These results are similar to previous studies with SAA patients treated with horse/rabbit ATG [33]. Most studies on horse/rabbit ATG also showed that patients with vSAA had higher morbidity due to infection and higher early mortality [34]. However, more prospective studies are required to validate our conclusions.

In summary, our retrospective results show the great potential of p-ALG plus CsA as the first-line IST in the treatment of acquired SAA. It shows similar efficacy to rabbit/horse ATG while the costs of treatment are substantially lower. Thus, it may prove to be a cheaper and more reliable alternative to rabbit ATG.

Table 5 Comparison of clinical efficacy of IST with different protocols in published data

Countries/study	Study type	Study number	ATG preparation	OS rate	OR rate (%)
China/current study	Retrospective	69	p-ALG	88.4 % (2 years)	76.8
China [28]	Retrospective	48	p-ALG	87.5 % (1.5 years)	83.3
USA [6]	Prospective	60	r-ATG	96 % (3 years)	37
		60	h-ATG	76 % (3 years)	68
Korea [25]	Retrospective	46	h-ATG	83.5 % (5 years)	45.7
		53	r-ATG	82.7 % (5 years)	49.1
Brazil [14]	Retrospective	42	h-ATG	78.4 % (2 years)	59.5
		29	r-ATG	55.4 % (2 years)	34.5
EBMT [8]	Prospective	105	h-ATG	86 % (2 years)	67
		35	r-ATG	68 % (2 years)	60
Japan [23]	Retrospective	12	r-ATG	NA	67
		14	h-ATG	NA	75

p-ALG porcine antilymphocyte globulin, *r-ATG* rabbit antithymocyte globulin, *h-ATG* horse antithymocyte globulin, *OS* overall survival, *OR* overall response, *NA* not available

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Conflict of interest All authors declare no conflicts of interest.

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Informed consent was obtained from all patients for being included in the study.

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抗淋巴细胞球蛋白联合环孢霉素A治疗重型再生障碍性贫血的临床研究

郑以州　储榆林　邵宗鸿　张益枝　陈桂彬

【摘要】　目的　探讨进一步提高重型再生障碍性贫血(SAA)疗效的方法。方法　采用前瞻性同期对照研究方法，比较抗淋巴细胞球蛋白(ALG)单用(NIIST方案)或联合环孢霉素A(CsA)(IIST方案)治疗SAA的疗效。结果　IIST方案组不仅疗效(83.7%)显著高于NIIST方案组(57.6%)，且可降低早期死亡率、缩短脱离红细胞输注时间，治疗有效者骨髓BFU-E和CFU-GM数量恢复更为良好。结论　ALG联合CsA治疗SAA疗效明显优于单用ALG，且治疗有效者骨髓造血功能恢复更为完全。

【关键词】　贫血，再生障碍性　抗淋巴细胞血清　环孢菌素　造血干细胞

Antilymphocyte globulin combining with cyclosporine A in the treatment of severe aplastic anemia

ZHENG Yizhou,CHU Yulin, SHAO Zonghong, et al. Institute of Hematology and Blood Diseases Hospital, CAMS and PUMC, Tianjin　300020

【Abstract】　Objective　To explore more effective regimen for severe aplastic anemia (SAA). Methods　A prospective, randomized clinical trial was conducted to determine whether the outcome of SAA patients treated with the combination of antilymphocyte globulin(ALG) and cyclosporine A(CsA) (intensive immunosuppressive therapy, IIST) was better than that with ALG alone (non-intensive immunosuppressive therapy, NIIST). Results　The response rate of IIST group(83.7%) was significantly higher than that of NIIST group(57.6%),with a lower risk of early mortality and a shorter time to red cell transfusion independence.Furthermore,the recovery of bone marrow BFU-E　and　CFU-GM of the responding patients was more complete in IIST group than in NIIST group.Conclusions　The treatment outcome was better with IIST than with NIIST for SAA.

【Key words】　Anemia, aplastic　Antilymphocyte serum　Cyclosporine　Hematopoietic stem cell

80年代我院应用国产抗胸腺细胞球蛋白(ATG)治疗重型再生障碍性贫血(SAA)并取得一定疗效[1]。为进一步探寻治疗SAA的新方案，我们于1991年5月～1996年8月对强化免疫抑制治疗(IIST)与非强化免疫抑制治疗(NIIST)，即抗淋巴细胞球蛋白(ALG)联合

或不联合环孢霉素A(CsA)治疗SAA的疗效进行同期对照研究，报道如下。

病例和方法

1　病例　76例SAA患者中，男54例，女22例，中位年龄34（2～73）岁;其中SAA-Ⅰ型61例,SAA-Ⅱ型15例，均为我院住院患者。76例SAA患者同期分为IIST及NIIST两组，两组患者在年龄、性别、SAA型别及病情严重程度等方面大致相同。

2　治疗方案

2.1　基础治疗药物：康力龙(18例)6～8 mg/d口服，或长效睾酮(58例)每次250 mg肌注，每周2次；Vit B_{12} 500 μg肌注，每周1次;叶酸30 mg/d，口服。以上用于全部病例(5例儿童患者上述药物剂量酌减)。

2.2　IIST方案(43例)：ALG(法国Pasteur Merieux血清与疫苗公司产品)12 $mg\cdot kg^{-1}\cdot d^{-1}$静脉输注，第1～5天；泼尼松1 $mg\cdot kg^{-1}\cdot d^{-1}$第1～15天（第1～5天静脉输注地塞米松及氢化可的松，按上述泼尼松剂量换算），如无严重血清病反应，第16天开始减量,至第30天停用；CsA（瑞士Novartis公司产品）3～5 $mg\cdot kg^{-1}\cdot d^{-1}$口服，第31天始用，3个月为1个疗程，根据血清肌酐及胆红素浓度调节其剂量。

2.3　NIIST方案（33例）：ALG单用，用法同上。

2.4　维持治疗：所有治疗有效病例，男性血红蛋白达到120 g/L，女性达到110 g/L时起继续用原方案（不包括IIST方案中的ALG）维持治疗2年或以上，维持治疗期间药物每3～6个月减量1次，并定期（3～6个月）随访病情变化，待病情达到“治愈”（骨髓造血祖细胞水平恢复正常）后方可完全停药。

2.5　以上两组患者均未加用造血细胞生长因子。

3　骨髓造血祖细胞体外培养　红系爆式集落形成单位(BFU-E)和粒-巨噬细胞集落形成单位(CFU-GM)均用甲基纤维素半固体培养法[2]。

4　随访观察项目　观察两组病例治疗前后血象、骨髓象、红细胞输注量及血小板输注量，以及感染频度及相关死亡情况、骨髓BFU-E及CFU-GM水平变化、末次随访时患者疗效。定期复查患者尿Rous试验、Ham试验、蛇毒因子溶血(CoF)试验、微量补体溶血敏感试验(mCLST)以及染色体核型和姐妹染色单体差异(SCD)等，以了解有无晚期并发症。

5　诊断及疗效标准　均按现行的全国统一标准[3]确定诊断及判断疗效。

6　统计学处理　两样本均数间比较用t检验，四格表资料用χ^2检验，生存率曲线的比较用Kaplan-Meier评估法。

结　　果

1　疗效

1.1　总有效率：76例SAA患者中，经治疗达到基本治愈36例(47.8%)，缓解9例(11.8%)，明显进步10例(13.1%)，无效21例(27.6%)，总有效率达72.4%。

1.2　两种治疗方案疗效比较：IIST组43例患者中达到基本治愈24例(55.8%)，缓解6例(14.0%)，明显进步6例(14.0%)，无效7例(16.3%)，总有效率为83.7%；NIIST组33例患者中达到基本治愈12例(36.4%)，缓解3例(9.1%)，明显进步4例(12.1%)，无效14例

(42.4%)，总有效率57.6%。IIST组各级别疗效及总有效率均高于NIIST组，且两组间基本治愈率及总有效率比较，差异具有显著性(χ^2=3.92,6.38,P均<0.05)。

2　两种方案治疗后患者造血功能恢复的比较

2.1　造血功能恢复速度的比较:

2.1.1　治疗期间两组患者严重感染频度及其相关死亡与中性粒细胞绝对值(ANC)>1.0×10^9/L的中位时间：两组患者治疗期间严重感染频度无明显差异，但NIIST组的感染相关死亡例数及病死率(21.2%)显著高于IIST组(7.0%)(χ^2=7.16,P<0.01)；此外，IIST组患者治疗后ANC>1.0×10^9/L的百分率(79.1%)亦显著高于NIIST组(45.5%)(χ^2=9.21, P<0.01)，但两组治疗有效患者ANC>1.0×10^9/L的中位时间十分相近(表1)。

2.1.2　脱离红细胞及血小板输注中位时间：IIST组患者脱离红细胞及血小板输注的百分率均显著高于NIIST组(χ^2=6.38,10.7,P分别<0.05及<0.01)，前者脱离红细胞输注中位时间亦明显短于后者，但两者脱离血小板输注中位时间相比，差异无显著性（表1）。

表1　两组患者严重感染频度,感染相关死亡例数及造血功能恢复速度的比较

组别	例数	严重感染频度(%)	感染相关死亡例数(%)	ANC>1.0×10^9/L		脱离红细胞输注		脱离血小板输注	
				例数(%)	中位时间(天数)	例数(%)	中位时间(天数)	例数(%)	中位时间(天数)
IIST组	43	14(32.6)	3(7.0)	34(79.1)	94(21～362)	36(83.7)	105(20～370)	34(79.1)	116(22～460)
NIIST组	33	15(45.5)	7(21.2)	15(45.5)	89(20～310)	19(57.6)	167(20～420)	14(42.4)	134(28～370)
χ^2值			7.16	9.21		6.38		10.70	
P值			<0.01	<0.01		<0.05		<0.01	

2.2　造血功能恢复程度的比较：所有患者治疗前后均定期复查骨髓BFU-E及CFU-GM数量变化。治疗前两组患者骨髓BFU-E及CFU-GM水平均显著低于正常对照组(BFU-E，两组与正常对照比较，t=4.36和4.12；CFU-GM，两组与正常对照比较，t=3.68和3.80,P均<0.001)；两组治疗无效者骨髓BFU-E及CFU-GM数量无明显增加(BFU-E两组无效者治疗前后比较，t=1.94和1.67；CFU-GM，两组治疗无效者治疗前后比较，t=1.50和1.95,P均>0.05)；两组治疗有效者骨髓BFU-E及CFU-GM数量均显著增加(BFU-E，两组治疗有效者治疗前后比，t=3.16和2.76；CFU-GM，两组治疗有效者治疗前后比，t=3.22和2.72,P均<0.01)，且IIST组较NIIST组增加更为明显(t=2.05和2.87,P<0.05和<0.01)，此与前者较高的基本治愈率相一致（表2）。值得注意的是，截至末次随访时间，两组治疗有效者中多数患者骨髓BFU-E及CFU-GM水平仍低于正常对照组，且两者恢复表现出不同步性。尽管如此，我们从检测结果发现IIST组中分别有12例(27.9%)及8例(18.6%)患者骨髓BFU-E及CFU-GM的数量恢复至正常范围；NIIST组中分别只有5例(15.1%)及3例(9.1%)患者骨髓BFU-E及CFU-GM水平恢复至正常范围。

表2　两组患者治疗前、后骨髓BFU-E、CFU-GM集落产率的变化($\bar{x}\pm s$)

组别	例数	BFU-E(个/2.0×10^4BMMNC$_5$)			CFU-GM(个/1.0×10^5BMMNC$_5$)		
		治疗前	治疗后		治疗前	治疗后	
			有效者	无效者		有效者	无效者
IIST组	43	3.1±1.6	24.6±13.6	5.9±2.4	4.3±2.1	21.1±8.2	5.6±3.4
NIIST组	33	3.3±1.9	13.6±7.1	4.8±2.9	3.9±2.0	11.6±5.0	6.0±3.8
正常对照组	20		33.6±10.2		30.1±14.3		

3　两组患者生存率的比较　IIST组早期(12个月内)死亡3例(死因均为败血症)，另4例成为输血依赖者；NIIST组早期死亡10例(7例死于败血症，2例死于颅内出血，另1例死于冠心病并发心室颤动)，另4例成为输血依赖者。中位随访时间64(27～88)个月，IIST组及NIIST组24个月生存率分别达93.0%及69.7%(χ^2=7.16,P<0.01)；根据Kaplan-Meier评估法计算，两组患者长期（60个月）生存率分别达83.7%及57.6%。

4　影响疗效因素　对两组疗效间差异的可能影响因素，包括患者性别、年龄、病因(特发性或继发性)、治疗前网织红细胞及血小板水平、初治或复治、治疗时出血及感染症状有无、病程、诊断至治疗间期等因素进行了分析，结果表明，上述诸因素均与两组间疗效的差异无相关性。

5　晚期并发症　截至末次随访时间，IIST组中1例获基本治愈者于18个月后复发，另1例输血依赖者并发血色病；NIIST组2例治疗有效者分别于14个月及17个月后复发，另有2例输血依赖者并发血色病。所有患者无一例发展为克隆性血液系统疾患。

讨　论

大量的临床及实验研究结果表明,异常免疫介导的造血抑制是SAA最常见的病理生理机制[4,5]。国内外学者早期研究表明，ALG/ATG单用治疗SAA疗效颇不一致，文献报道其疗效为28%～67%。我院80年代应用国产ATG联合康力龙治疗23例SAA患者，基本治愈加缓解率达30.4%，总有效率为52.2%，1年生存率达50.0%。如何在ALG/ATG联合雄激素基础上进一步提高SAA的疗效，尤其是避免ALG/ATG治疗早期过程中感染相关性死亡及提高临床治愈率一直是各国学者苦苦探索的难题。

激活的T淋巴细胞及其释放的某些过量的造血负调控因子可能是SAA发病机制方面最为重要的环节[6]。作为特异性免疫抑制剂，CsA可通过阻断白细胞介素2受体(IL-2R)来抑制T淋巴细胞被激活并进而抑制其释放过量的造血负调控因子，如IL-2、γ干扰素(IFN-γ）及肿瘤坏死因子(TNF)-α等[7]，且我们临床研究证明CsA单用治疗SAA具有一定疗效[8]。为明确ALG与CsA治疗SAA有无协同效应，我们采取前瞻性同期对照研究方法，比较了ALG联合CsA治疗SAA的疗效，结果表明，ALG联合CsA这一IIST方案治疗SAA的疗效颇为令人鼓舞，其疗效显著高于NIIST方案组，并可降低早期死亡率及缩短脱离红细胞输注时间，而且长期随访观察表明,IIST方案组有效者骨髓造血祖细胞恢复更为良好。

另外，与国外文献报道相比，本组患者复发率较低，随访27～88个月，仅3例(3.9%)复发，且无一例发展为克隆性血液系统疾患，此可能与我们采取较长时间维持治疗及更为严格的诊断措施有关。

作者单位:郑以州　储榆林　邵宗鸿　张益枝　陈桂彬　300020　天津,中国医学科学院、中国协和医科大学血液学研究所、血液病医院

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(校对：张志方)

国产抗人T细胞猪免疫球蛋白联合环孢素治疗再生障碍性贫血的临床疗效观察

陈思言，杨丽华，董 事，何吉琼
（达州市中心医院血液内科，四川 达州 635000）

【摘要】目的 研究再生障碍性贫血患者实施抗人T细胞猪免疫球蛋白联合环孢素治疗临床的价值。方法 纳入本院（2014年04月～2018年10月）接收的再生障碍性贫血患者（n=86）作为研究对象进行研究，以随机表为基准，分为实验组（n=43，实施国产抗人T细胞猪免疫球蛋白+环孢素治疗）、对照组（n=43，实施国产抗人T细胞猪免疫球蛋白治疗），对86例患者临床总有效率、起效时间、不良反应发生率、血常规指标评估。结果 临床总有效率：实验组高于对照组，P＜0.05。起效时间：实验组低于对照组，P＜0.05。不良反应发生率：实验组低于对照组，P＜0.05。血常规指标：实验组优于对照组，P＜0.05。结论 再生障碍性贫血患者实施国产抗人T细胞猪免疫球蛋白联合环孢素治疗临床的价值显著，既可改善临床血常规指标，又可提高治疗安全性，值得借鉴。

【关键词】再生障碍性贫血；国产抗人T细胞猪免疫球蛋白；环孢素；起效时间；造血功能
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Clinical observation of domestic anti-human T cell porcine immunoglobulin combined with cyclosporine in the treatment of aplastic anemia

CHEN si-yan,YANG Li-hua,DONG Shi,HE Ji-qiong
(Department of hematology,Dazhou central hospital,Sichuan Dazhou 635000,China)

【**Abstract**】**Objective** To investigate the clinical value of anti-human T cell porcine immunoglobulin combined with cyclosporine in the treatment of aplastic anemia.**Methods** In our college (04,2014 - October 2018) received aplastic anemia patients (n = 86) as the research object,based on random table,divided into experimental group (n=43,the implementation of domestic pig immunoglobulin + T cells ring spore (treatment),the control group (n=43,implementation of domestic human T cells pig immunoglobulin treatment resistance),86 cases of patients with clinical total effective rate,work time and incidence of adverse reactions,routine blood index evaluation. **Results** The total clinical effective rate in the experimental group was higher than that in the control group,P＜0.05. Effective time:the experimental group was lower than the control group,P＜0.05.Incidence of adverse reactions:the experimental group was lower than the control group,P＜0.05.Blood routine indexes: the experimental group was superior to the control group,P＜0.05.**Conclusion** The clinical value of domestic anti-human T cell porcine immunoglobulin combined with cyclosporine in the treatment of aplastic anemia patients is significant,which can not only improve the clinical blood routine indicators,but also improve the safety of treatment.

【**Key words**】Aplastic anemia;Domestic anti-human T cell porcine immunoglobulin;Ring spore element;Effective time;Hematopoietic function
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再生障碍性贫血是药物、化学毒物、辐射、病毒感染、免疫因素、遗传等因素诱发的骨髓造血功能障碍性疾病，该项疾病好发于青状年人群，且男性发病率高于女性，临床以外周血细胞减少、骨髓增生不良、感染、出血等为临床表现，对患者生命安全影响较为显著[1]。临床多其多实施免疫抑制法进行治疗，常见抗人T细胞猪免疫球蛋白、环孢素两种药物治疗，临床数据显示[2]，将免疫抑制法应用于缺少人白细胞抗原障碍性贫血患者中有一定价值，可使临床2/3人群临床症状得到有效改善。免疫抑制法通过清除血液循环中异常活化的免疫细胞，使补体所依赖的细胞得到溶解，出现凋亡，故本次随机选择86例再生障碍性贫血患者，实施猪抗人胸腺淋巴细胞球蛋白+环孢素联合治疗，对其治疗后血常规水平、不良反应发生率进行研究，现将具体研究结果汇总如下。

1 资料与方法

1.1 一般资料

纳入本院（2014年04月～2018年10月）接收的再生障碍性贫血患者（n=86）作为研究对象进行研究，分为2组（随机表），即每组样本数：43例。

实验组：男：28例，女：15例；年龄：18～52（平均：35.0±17.0）岁；病程：0.5～7.5（平均：4.0±3.5）月；

对照组：男：29例，女：14例；年龄：20～56（平均：38.0±18.0）岁；病程：0.3～8（平均：4.1±3.8）月。细致分析、对比2组（n=86）基础资料，差异显著P＞0.05，具有可比性。该研究上报医院伦理委员会，并获得相关人员审批，本研究遵循自愿、保密、尊重三大原则，凡涉及患者隐私相关内容信息，均严格遵循相关原则，维护患者自身权益，且患者自愿签署知情同意书。

排除：（1）药物过敏患者；（2）依从性较差患者；（3）肝、肾功能异常患者；（4）病毒感染史患者；（5）恶性肿瘤患者；（6）骨髓增生异常患者；（7）范可尼贫血患者；（8）脾功能亢进患者；（9）体温异常高热患者。

纳入：（1）配合能力较好；（2）无药物过敏患者；（3）家属知情，且自愿参加治疗患者；（4）临床资料齐全患者；（5）治疗前未接受骨髓移植患者；（6）无病毒

感染史患者。

注：研究需严格遵循排纳标准及三大原则，避免降低临床参考价值，引起临床医患纠纷。

1.2 方法

实验组：实施国产抗人T细胞猪免疫球蛋白+环孢素（国药准字：S10830001；生产企业：武汉生物制品研究所有限责任公司；规格：50ml：5g；）治疗，待感染控制后实施环孢素治疗，初始剂量：3～5 mg/kg/天，2次/天，治疗6-12月，用药后7天进行血药浓度测定，将环孢素血药浓度峰控制在200～400 ng/ml，以确保治疗效果。国产抗人T细胞猪免疫球蛋白：首次剂量：0.4 mg/kg/d，1次/天，治疗3～5天，此后28天治疗1次，治疗6～8次。

对照组：实施猪抗人胸腺淋巴细胞球蛋白（批准文号：S-19990003；生产企业：Fresenius AG，Immune Therapy Division；）治疗，首次剂量：0.4 mg/kg/d，1次/天，治疗3～5天，此后28天治疗1次，治疗6～8次。

支持治疗：治疗前对感染病灶进行清除，并协助患者做好口腔、皮肤、会阴等部位清洁工作，可指导患者口服抗生素，增强机体抵抗能力，并指导患者进食无菌饮食[3]。

1.3 分析指标

1.3.1 临床总有效率评估

参考血液血标准评价，（1）显效：血红蛋白恢复至正常范围，血小板范围在150×10^9/L以上，且中性粒细胞范围在1.5×10^9/L以上；（2）有效：监测显示血红蛋白、血小板、中性粒细胞均有一定改善，且不在再生障碍性贫血诊断范围内，无需进行血液输注治疗；（3）无效：患者需进行血液输注治疗，且监测显示血红蛋白、血小板、中性粒细胞无显著改善[4]。

1.3.2 起效时间评估

对86例患者治疗后起效时间评估。

1.3.3 不良反应发生率评估

常见牙龈增生、手颤、多毛、毛囊炎、疱疹、肾功能损害、继发性糖尿病等。

1.3.4 血常规指标评估

采用血液分析仪对患者血红蛋白、血小板、中性粒细胞3项血常规指标评估。

1.4 统计学方法

SPSS 22.0，计量资料（包括：起效时间、血常规指标）用t、平方差[（$\bar{x}\pm s$）]进行检验、表示，计数资料（包括：临床总有效率、不良反应发生率）以卡方（x^2）、百分数（%）进行检验、表示，2组数据资料，以P值区间（$P<0.05$）表示统计学差异。

2 结 果

2.1 临床总有效率

86例患者临床总有效率评估，详见表1。数据显示：实验组临床总有效率（83.72%）高于对照组，$P<0.05$。

表1 86例患者临床总有效率评估 [n（%）]

组别	n	显效	有效	无效	总有效率
实验组	43	20（46.51）	16（37.21）	7（16.28）	36（83.72）
对照组	43	15（34.88）	12（27.92）	16（37.21）	27（62.79）
x^2	--	--	--	--	4.8075
P	--	--	--	--	0.0283

2.2 起效时间

86例患者起效时间评估，详见表2。数据显示：实验组起效时间（41.76±3.91d）优于对照组，$P<0.05$。

2.3 不良反应发生率

86例患者不良反应发生率评估，详见表3。数据显示：实验组牙龈增生不良反应发生率高于对照组，$P<0.05$。其余不良反应对比无差异，给予环孢素减量后均得到有效缓解。

表2 86例患者起效时间评估（$\bar{x}\pm s$）

组别	n	起效时间（d）
实验组	43	41.76±3.91
对照组	43	72.61±6.54
t	--	26.5492
P	--	0.0000

表3 86例患者不良反应发生率评估 [n（%）]

组别	n	牙龈增生	手颤	多毛	毛囊炎	疱疹	肾功能损害	继发性糖尿病
实验组	43	7（16.28）	2（4.65）	3（6.98）	6（13.95）	7（16.28）	2（4.65）	3（6.98）
对照组	43	1（2.32）	0（0.00）	0（0.00）	5（11.63）	4（9.30）	3（6.98）	1（2.32）
x^2	--	4.9615	2.0476	3.1084	0.1042	0.9382	1.9779	1.0488
P	--	0.0259	0.1524	0.0779	0.7468	0.3327	0.1596	0.3058

2.4 血常规指标

86例患者血常规指标评估，详见表4。数据显示：实验组血常规指标优于对照组，$P<0.05$。

表3 86例患者血常规指标评估（$\bar{x}\pm s$）

组别	n	白细胞（$\times10^9$/L）	血小板（$\times10^9$/L）	中性粒细胞（g/L）
实验组	43	4.86±0.62	96.42±15.25	51.75±12.31
对照组	43	3.31±0.42	71.25±15.31	38.55±10.02
t	--	13.5726	7.6380	5.4533
P	--	0.0000	0.0000	0.0000

3 讨 论

再生障碍性贫血属于免疫介导性疾病，临床医师多建议实施骨髓抑制治疗，但其不仅配型较为困难，且手术费用较高，大部分患者无法承受此经济压力。经查阅学术性文献发现[5]，免疫抑制法在治疗再生障碍性贫血中有一定价值，其通过抗人T细胞猪免疫球蛋白、环孢素两种药物的联合，改善患者临床症状、血常规指标，提高治疗效率。

抗人T细胞猪免疫球蛋白通过清除T细胞对骨髓造血的抑制，调节免疫刺激促进血液内造血干细胞的繁殖，但起效较慢。环孢素为临床常见免疫抑制剂，其口服后通过阻断白介素-2受体的表达，对毒性细胞的增殖进行干预，从而抑制T细胞产生白介素-2、干扰素，到达治疗目的，但其单一使用效果不太明显，故将两种药物联合进行治疗，通过抑制T细胞，降低其对造血因子（负性因子）的调控，逐渐解除其对造血干细胞的破坏，实现造血干细胞的重建、增殖，达到治疗目的[6]。本次研究中，实验组临床总有效率为83.72%高于对照组，起效时间为41.76±3.91 d优于对照组，且3项血常规指标优于对照组，该研究结果与谷月丽[7]等相似性较高，谷月丽研究证实猪抗人胸腺淋巴细胞球蛋白+环孢素联合治疗应用于再生障碍性贫血治疗中，不仅药物起效时间较快，且血常规水平恢复较好，这与本次研究一致，故抗人T细胞猪免疫球蛋白+环孢素联合治疗价值得到证实。药物联合治疗过程中不良反应发生率较高，但经环孢素减量后均得到有效缓解，且国外进口猪抗人胸腺淋巴细胞球蛋白价格较高，随着我国医疗技术的发展，国内猪抗人胸腺淋巴细胞球蛋白逐渐应用于临床，且价格较低，在临床联合治疗中，不仅可提高治疗效果，还可降低患者经济压力。

综合上述，再生障碍性贫血患者实施猪抗人胸腺淋巴细胞球蛋白+环孢素联合治疗，既可改善临床血常规水平，又可缩短起效时间，值得借鉴。但基于本次研究时间较短、样本数量选取范围有限，未对治疗后复发率、生存率数据进行采集、评估，故后期研究中需加大研究范围及样本采集数量，通过对复发率、生存率等数据的综合分析，为临床治疗提供更高参考。

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·医学研究·

猪抗淋巴细胞免疫球蛋白联合环孢素治疗重型再生障碍性贫血疗效观察

谷月丽

商丘市第一人民医院 血液科，商丘 476000

摘要：目的 观察猪抗淋巴细胞免疫球蛋白联合环孢素治疗重型再生障碍性贫血（SAA）方面的临床疗效。**方法** 选择商丘市第一人民医院自 2009 年 10 月至 2013 年 10 月收治的 41 例 SAA 患者的临床资料，治疗组患者采用猪抗淋巴细胞免疫球蛋白联合环孢素，对照组患者只给予猪抗淋巴细胞免疫球蛋白治疗。**结果** 用药 1 年后，两组患者临床有效率比较，结果：具有显著性差异（$P<0.05$），治疗前，两组患者 WBC、HGB 及 PLT 指标无显著性差异（$P>0.05$），但用药 1 年后，治疗组患者上述指标明显高于对照组患者，结果比较，具有显著性差异（$P<0.05$），治疗组患者平均起效时间明显低于对照组患者（$P<0.05$）。治疗期间，治疗组患发生牙龈增生、手颤、继发性糖尿病患者明显高于对照组患者（$P<0.05$），毛囊炎、多毛、带状疱疹及肝功能受损发生率比较，无显著性差异（$P>0.05$）。**结论** 从近期疗效看来，采用猪抗淋巴细胞免疫球蛋白联合环孢素治疗 SAA 临床疗效高，其安全性仍需进一步研究确认。

关键词： 猪抗淋巴细胞免疫球蛋白；环孢素；重症再生障碍性贫血

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SAA 是一种获得性骨髓造血功能衰退，表现为外周全血细胞减少，骨髓增生不良，主要临床表现为贫血、感染及出血症状，患者预后凶险[1]。采用免疫掏治疗 SAA 是重要的治疗方案，目前，采用免疫球蛋白联合环孢素是 SAA 的标准治疗方法[2]。联合用药可降低免疫抑制剂的剂量，提高耐受力，降低药物毒副反应[3]。本组研究中，采用猪抗淋巴细胞免疫球蛋白联合环孢素治疗 SAA，观察其临床疗效及患者不良反应，旨在为临床用药提供参考，现报告如下。

1 资料与方法

1.1 **一般资料** 选择自 2009 年 10 月至 2013 年 10 月收治的 41 例 SAA 患者的临床资料，患者中男性 25 例，女性 16 例；患者年龄 16～50 岁，平均年龄（29.7±4.8）岁；患者病程 0.5 个月～8 个月，平均病程（1.9±0.4）个月。根据随机的原则，将患者分为治疗组 21 例与对照组 20 例，两组患者在性别、年龄、病情等方面比较，无显著性差异（$P>0.05$），具有可比性。

1.2 **病例纳入标准** 根据《血液病诊断及疗效标准（第 3 版）》中关于 SAA 的诊断，科学出版社，张之南，沈悌主编，

1.3 **排除病例标准** 阵发性睡眠性血红蛋白尿、骨髓增生异常综合征、脾功能亢进、范可尼贫血；肝肾功能严重障碍；有病毒感染史。

1.4 **方法** 治疗组患者采用猪抗淋巴细胞免疫球蛋白联合环孢素，猪抗淋巴细胞免疫球蛋白首次用量 0.4 mg/（kg·d），连续服用 3～5d，此后 1 次/28d，服用 6～8 次，感染控制后给予环孢素 A，初始用量 3～5mg/（kg·d），分两次口服，如明显起效，减量为 0.5～2 mg/（kg·d），持续治疗 6 个月～1 年，用药第 1 周起做环孢素 A 血药浓度测定，将血药浓度控制在 200ng/mL～400ng/mL。对照组患者只给予猪抗淋巴细胞免疫球蛋白治疗，用法与用量同治疗组患者。观察两组患者用药的临床疗效及用药期间的不良反应发生情况。

1.5 **疗效判定标准** 基本治愈：脱离输血后，患者血红蛋白（HGB），男性 >120/L，女性 >110g/L，白细胞（WBC）$>1.5\times10^9$/L，白小板计数（PLT）$>100\times10^9$/L。明显缓解：脱离输血后，HGB >80g/L，PLT $>30\times10^9$/L；改善：各项标准不再符合 SAA 标准，或脱离红细胞或血小板输注；无效：以上改善皆无。有效率 =（基本治愈例数 + 明显缓解例数 + 改善例数）/总例数 ×100%。起效时间：外周血网织红细胞 >1%。

1.6 **统计学方法** 使用 SPSS 10.0 统计软件包进行统计分析，计量资料以（$\bar{x}\pm s$）表示，采用 χ^2 检验、t 检验处理数据。$P<0.05$ 具有显著性差异。

2 结 果

用药 1 年后，两组患者临床有效率比较，结果具有显著性差异（$P < 0.05$），详见下表 1。治疗前，两组患者 WBC、HGB 及 PLT 指标无显著性差异（$P > 0.05$），但用药 1 年后，治疗组患者上述指标明显高于对照组患者，结果比较，具有显著性差异（$P < 0.05$），详见下表 2。患者起效平均时间（41.5 ±4.8）d，对照组平均起效时间（72.5 ±6.1）d，治疗组患者平均起效时间明显低于对照组患者（$P < 0.05$）。治疗期间，治疗组患发生牙龈增生、手颤、继发性糖尿病患者明显高于对照组患者，毛囊炎、多毛、带状疱疹及肝功能受损发生率比较，无显著性差异，部分患者合并两种症状，全部患者均环孢素减量应用后，并发症得到好转，详见下表 3。

表 1 两组患者临床有效率比较情况表

组别	例数	基本治愈	明显缓解	改善	无效	有效率（%）
治疗组	21	3	7	6	5	76.2[a]
对照组	20	1	5	4	10	50.0

注：与对照组比较，[a]$P < 0.05$。

表 2 两组患者外周血常规比较情况表

组别	例数	WBC（$\times 10^9$/L）		HGB（g/L）		PLT（$\times 10^9$/L）	
		治疗前	治疗后	治疗前	治疗后	治疗前	治疗后
治疗组	21	2.4 ±0.6	4.8 ±0.6[a]	52.1 ±7.6	96.5 ±10.5[a]	32.1 ±12.0	51.8 ±12.5[a]
对照组	20	2.3 ±0.5	3.3 ±0.5	51.9 ±7.8	71.3 ±11.4	31.9 ±11.8	39.5 ±11.1

注：与对照组比较，[a]$P < 0.05$。

表 3 两组患者副反应发生情况表（例）

组别	例数	牙龈增生	手颤	继发性糖尿病	毛囊炎	多毛	带状疱疹	肝功能受损
治疗组	21	7	3	2	4	5	4	3
对照组	20	1	0	0	4	6	4	4

3 讨 论

采用淋巴细胞免疫球蛋白联合环孢素 A 是治疗 SAA 的标准方案，传统多采用进口马或兔抗淋巴细胞免疫球蛋白治疗，其造价较高，给患者带来了沉重的负担[4]。

本组中，通过采用猪抗淋巴细胞免疫球蛋白与环孢素联合治疗 SAA，观察其临床疗效。观察发现，采用国产猪抗淋巴细胞免疫球蛋白联合环孢素 A 治疗 SAA 其临床有效率明显高于单纯应用猪抗淋巴细胞免疫球蛋白治疗的患者，且患者外周血常规指标改善情况、起效时间方面也明显优于对照组患者，表明联合用药具有起效快，疗效佳的优势[5]。但研究不难发现，患者发生副反应的例次也高于单纯用药的患者。分析认为，与环孢素用量有一定关系，在给予减量应用后，患者的症状明显好转。表明猪抗淋巴细胞免疫球蛋白治疗的用药耐受性良好，安全性较高。临床研究指出，采用联合用药可能增加急性髓细胞白血病等克隆性疾病的发生，但本组研究并未发现此项病例，分析认为可能与病例数量少及研究年限短等因素有关，其安全性仍需进一步研究确认[6]。

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国产猪抗人淋巴细胞球蛋白联合环孢素治疗重型再生障碍性贫血的效果

薛连国　毛建平　贾　韬　王　莹　陈　泽　蔡志梅▲　赵利东
江苏省连云港市第一人民医院血液科，江苏连云港　222002

[摘要] 目的 探讨国产猪抗人淋巴细胞球蛋白(p–ALG)联合环孢素治疗重型再生障碍性贫血(SAA)的效果及安全性。方法 分析2004年4月~2016年12月江苏省连云港市第一人民医院血液科应用国产p–ALG联合环孢素治疗30例SAA患者的有效性、安全性并长期随访统计分析五年生存率。结果 30例SAA患者中位年龄为26.5(5~65)岁，随访中位时间为60(1~252)个月，6例死亡，总有效率为73.3%，中性粒细胞>0.5×10⁹/L，中位时间为20(2~40)d，脱离红细胞输注的中位时间为30(0~150)d，脱离血小板输注的中位时间为35.5(5~140)d。五年生存率达78%，不良反应均能耐受，花费少。结论 国产p–ALG联合环孢素治疗SAA的长期效果显著，不良反应轻微，值得临床推广。

[关键词] 重型再生障碍性贫血；猪抗人淋巴细胞球蛋白；环孢素；效果
[中图分类号] R556　　[文献标识码] A　　[文章编号] 1673–7210(2017)07(b)–0078–04

Effect of Pig Anti–Human Lymphocyte Globulin combined with Cyclosporin in the treatment of severe aplastic anemia

XUE Lianguo　MAO Jianping　JIA Tao　WANG Ying　CHEN Ze　CAI Zhimei▲　ZHAO Lidong
Department of Hematology, the First People's Hospital of Lianyungang City, Jiangsu Province, Lianyungang　222002, China

[Abstract] Objective To analyze the efficacy and safety of Pig Anti–Human Lymphocyte Globulin combined with Cyclosporin in the treatment of severe aplastic anemia. **Methods** A total of 30 cases hospitalized in Department of Hematology, the First People's Hospital of Lianyungang City, Jiangsu Province from Apirl 2004 and December 2016 were enrolled into this study. The efficacy and safety of this treatment were analyzed, the overall survival by Kaplan–Meier method for this cohort of patients were estimated. **Results** The cohort of 30 patients was routinely followed up with a median follow–up of 60 (1–252) months, the median age was 26.5 (5–65) years old. Six died, the over response rate was 73.3%. The median time of the ANC>0.5×10⁹/L was 20 (2–40) days, transfusion of red blood cell independent was 30 (0–150) day, transfusion of palete independent was 30 (0–150) days, the 5–year survival rate was 78%. The response rate was high, but with less side effects and expense. **Conclusion** Long–term effect of Pig Anti–Human Lymphocyte Globulin combined with Cyclosporin in the treatment of severe aplastic anemia is significant, adverse reactions are mild, it is worthy of clinical promotion.

[Key words] Severe aplastic anemia; Pig Anti–Human Lymphocyte Globulin; Cyclosporin; Effect

重型再生障碍性贫血(SAA)是由多种病因、多种发病机制导致的一种获得性骨髓造血功能衰竭综合征。目前认为T淋巴细胞异常活化、功能亢进造成骨髓损伤、造血细胞凋亡和造血功能衰竭在SAA发病机制中占主要地位，其中免疫功能异常被临床认为是AA最主要的发病机制[1]。对年龄≥40岁或年龄虽<40岁，但无HLA相合同胞供者的SAA患者首选抗人胸腺细胞球蛋白(ATG)/猪抗人淋巴细胞球蛋白(p–ALG)联合环孢素(CsA)的强化免疫抑制(IST)加促造血治疗[2]。目前临床上ATG治疗SAA应用广泛，报道较多，但p–ALG治疗SAA报道相对少见。江苏省连云港市第一人民医院(以下简称"我院")2004年4月开始应用p–ALG联合CsA治疗SAA，现将近期及长期疗效、并发症及不良反应总结报道如下。

▲通讯作者

1 资料与方法

1.1 一般资料

2004年4月~2016年12月应用p–ALG联合CsA治疗我院血液科收治的SAA患者30例。治疗前均明确诊断及分型，依据Ham试验、外周血粒细胞及成熟红细胞CD55/CD59表达水平、骨髓细胞形态学、细胞遗传学、骨髓病理及免疫学相关检查排除急性造

血功能停滞、阵发性睡眠性血红蛋白尿(PNH)、骨髓增生异常综合征、骨髓纤维化、免疫性全血细胞减少、急性白血病等疾病,诊断标准参照《血液病诊断及疗效标准》[3]。

1.2 治疗方法

所有患者均入住层流病房,接受 p–ALG(武汉生物制品研究所)治疗,第 1~5 天。治疗前常规皮试,皮试反应阴性者给予 15~20 mg/(kg·d)静脉输注,先以 10 mg ALG 加入生理盐水 500 mL 中缓慢静滴 6 h,如无反应,当日余量缓慢滴入;用药前 30 min 口服异丙嗪 25 mg 及甲泼尼龙 40 mg/d(第 1~5 天),应用地塞米松 10 mg/d 静脉滴注同步维持预防变态反应,如无严重的血清病反应第 6 天开始减量,至第 30 天停用。CsA 3~5 mg/(kg·d)口服,第 31 天开始使用,根据 CsA 血药浓度、肝肾功能情况调整剂量,维持至少 1 年以上。康力龙 6 mg/d 或安特尔 80~120 mg/d (2~3 次口服),连续 3 个月以上,同时给予护肝治疗,定期复查肝功能。

支持治疗:治疗前排查感染病灶并清除,做好口腔、皮肤、会阴等部位的护理,口服肠道不吸收抗生素以及氟康唑,无菌饮食。根据病情需要输注红细胞及血小板,条件允许时使用粒细胞集落刺激因子(G–CSF)、粒细胞–巨噬细胞集落刺激因子(GM–CSF)、重组人红细胞生成素(rhEPO)及重组人白介素–11(rhIL–11)。有感染者选用敏感抗生素,根据具体的药敏随时调整用药。

1.3 疗效评价标准

采用英国血液学标准委员会标准[4]判定疗效:①血红蛋白达同龄人正常水平,血小板>150×10⁹/L,中性粒细胞>1.5×10⁹/L,为完全治疗反应(CR);②脱离输注血制品,血液学检查好转,不再符合 SAA 标准,为部分治疗反应(PR);③未脱离血制品输注支持治疗,血液学检查仍符合 SAA 标准为无治疗反应(NR)。有效=CR+PR。复发是指患者血常规再次下降至需要输血,或者需要再次强化免疫抑制治疗及造血干细胞移植[5]。克隆性演变指 SAA 转化为血液学克隆性疾病,主要包括 PNH、MDS、AML。早期死亡是指 ATG 治疗后 60 d 内死亡[6]。

1.4 随访

定期复查血常规、生化、CsA 浓度,监测 CD55、CD59,尿 Rous 试验,血 Ham 试验等,必要时复查骨髓细胞形态、骨髓染色体核型评价疗效并了解有无晚期并发症,有无恶性克隆性疾病的发生。

1.5 统计学方法

采用 SPSS 17.0 统计学软件进行分析,Kaplan–Meier 评估法绘制生存曲线。

2 结果

2.1 患者临床基本特征

30 例 SAA 患者接受 p–ALG 联合 CsA 治疗,其中男 21 例,女 10 例,中位年龄为 26.5(5~65)岁,自诊断至接受 IST 治疗中位时间为 18 d(2 d~2.5 年)。发病时中性粒细胞中位值为 0.34(0~3.49)×10⁹/L,Hb 中位值 69(37~145)g/L,血小板中位值为 7(1~28)×10⁹/L。患者中 29 例为原发性 SAA,1 例为肝炎相关 SAA,其中 1 例病程 2.5 年,p–ALG 治疗前已接受 CsA、雄激素及对症支持治疗,余患者在强化免疫治疗前未接受相关治疗。29 例患者均接受 1 个疗程 p–ALG 治疗,1 例在 p–ALG 治疗 0.5 年后未见效果转外院接受 ATG 治疗。

2.2 早期死亡

30 例 SAA 患者中,6 例死亡,占所有患者的 20%,其中 1 例早期死亡,为 vSAA 患者,于治疗 0.5 个月后死于重度肺部感染;3 例治疗无效分别于治疗后 4、4、12 个月死于严重感染,1 例治疗后获 PR,3 年后转为 PNH 未再进一步治疗而死亡,1 例治疗 2 个月后并发溶血,因家庭矛盾及患者心理抵抗力弱自杀死亡。

2.3 近期疗效及血液学反应

12 例患者经 1 个疗程 p–ALG 治疗后取得 CR,占所有患者的 40%,10 例 PR(33.3%),总有效率为 73.3%,2 例 NR(6.7%),其中 1 例治疗 0.5 年后于外院接受 ATG 治疗,获得 PR。治疗有效患者中性粒细胞>0.5×10⁹/L 的中位时间为 20 (2~40)d, 中性粒细胞>1×10⁹/L 中位时间为 26.5(3~50)d,脱离红细胞输注的中位时间为 30(0~150)d,脱离血小板输注的中位时间为 35.5(5~140)d。

2.4 复发及长期生存率

随访中位时间为 60(1~252)个月,随访至今,30 例患者中有 1 例失访,6 例死亡。23 例仍存活,5 年累积生存率为 78%(图 1)。复发 1 例,1 例治疗后 3 年转为 PNH, 未见转化为急性白血病及骨髓增生异常综合征等恶性克隆性疾病的病例。

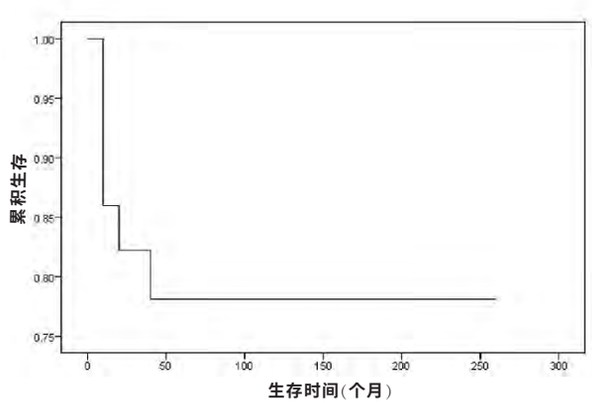

图 1 患者累积生存情况

2.5 不良反应

所有 p–ALG 联合 CsA 治疗的 30 例患者中，6 例发生血清病反应，多发生于 ALG 应用 1~2 周后，5 例症状较轻微，表现为发热、皮疹、水肿、关节痛，经适当对症治疗后好转；1 例病情较重，明显水肿，出现腹腔、胸腔及心包多浆膜腔积液，伴明显腹胀，经大剂量甲泼尼龙冲击治疗后好转。由于 ALG 应用前及用药过程中均采取严密抗过敏措施，无变态反应发生。2 例发生轻度肝功异常，17 例出现不同程度的多毛症及齿龈增生，考虑与联合应用 CsA 相关，经积极保肝降酶及调整 CsA 剂量治疗后好转。发生溶血 1 例，无肾功能损害。血小板<10×10^9/L 时积极予以 60 钴照射 25 Gy 的新鲜血小板或应用血小板滤器输注血小板，避免内脏出血的发生，未发生心脑血管意外。

3 讨论

CsA 作为常用的免疫抑制药物治疗 AA 的机制是阻断白介素–2（IL–2）受体的表达从而阻止细胞毒性 T 细胞的激活和增殖，抑制 T 淋巴细胞产生 IL–2 和 γ 干扰素，单用 CsA 治疗 AA 的有效率为 50%~60% [7]；而 ATG/ALG 作为目前公认治疗 SAA 的有效药物是通过去除抑制性 T 细胞对骨髓造血的抑制，以及通过免疫刺激作用产生一些造血调控因子促进干细胞增殖，以及对造血干细胞本身可能的直接刺激作用，有效率为 50%~70%；单独应用 CsA 或 ATG/ALG 起效慢，一般都在 3 个月以后[8–9]。研究显示，ATG 联合 CsA 治疗 SAA 的免疫抑制疗法效果明显优于单用 ATG 或 CsA[10]，并且已作为无法进行异基因造血干细胞移植及年龄>40 岁患者的一线治疗选择[11–12]。ATG/抗人淋巴细胞球蛋白和 CsA 为主的这种联合免疫抑制治疗的总机制是抑制 T 淋巴细胞，降低其所产生的负性造血调控因子，并解除这类造血负调控因子对造血细胞的抑制剂破坏，从而实现造血重建[13]。

在西方国家，SAA 初次标准免疫抑制疗法是马 ATG 联合 CsA，有效率能达到 60%~70%，并取得理想的长期生存率，同样在美国、欧洲和日本一些回顾性研究中也取得相似的疗效[14–17]。国外曾普遍以马–ATG（H–ATG）作为首选剂型，将兔–ATG（R–ATG）应用于 H–ATG 无效或复发患者的第二次 IST 治疗，但是由于 H–ATG 供应不足，且 R–ATG 作为首选剂型效果相当，所以目前国外已将 R–ATG 作为首选剂型。

2004 年以前在中国只有 R–ATG 可以应用，但是由于价格十分昂贵，导致许多患者因无法承担高额的治疗费用错失治疗良机。近年来我国的一些医学中心开始应用 p–ATG 替代 R–ATG 进行免疫抑制治疗，取得与利用马或兔治疗 SAA 相当的疗效，花费仅为进口 ATG 的 1/3，但这方面研究国内外报道仍较少。

本研究中应用 p–ATG 联合 CsA 治疗 30 例 SAA，总有效率 73.3%，与梁冬梅等[18]应用兔/马 ATG 联合 CsA 治疗 18 例 SAA 患者的总有效率（72.2%）相当，稍低于曹慧敏等[19]对 76 例 SAA 应用兔 ATG 联合 CsA 治疗的有效率（82.2%）及张兴霞等[20]报道的猪 ALG 治疗的 SAA 患者的有效率（81.8%）。本研究结果显示，治疗有效患者中性粒细胞>0.5×10^9/L 中位时间为 20（2~40）d，脱离红细胞输注的中位时间为 30（0~150）d，脱离血小板输注的中位时间为 35.5（5~140）d。而上述曹慧敏等[19]的研究显示，脱离红细胞输注的中位时间为 55（0~210）d，脱离血小板输注的中位时间为 49（2~166）d，与本研究基本一致。由于样本量偏少，仍需要继续随访并总结更多病例进一步证实。通过观察发现，脱离输血最长的患者达 5 个月，部分 3 个月后方见到疗效，所以临床中应有足够耐心，并与患者及家属做好沟通，坚持治疗，不能轻易放弃。

本研究通过生存分析显示，p–ALG 联合 CsA 治疗 SAA 的累积 5 年生存率达 78%，与 Chen 等[21]报道的 p–ALG 治疗 SAA 5 年生存率（81.8%）相当。6 例死亡患者多死于感染，因治疗后中性粒细胞无反应且长期处于粒细胞缺乏状态，且均病程超过 1 个月，治疗不及时，所以应早发现早治疗，否则延误病情，影响疗效，甚至出现严重并发症。

p–ALG 临床应用中常见的不良反应有血清病、变态反应、肝功能异常、自身免疫性溶血性贫血，以及中性粒细胞缺乏继发感染、血小板减少合并出血[22]。本研究中有 6 例发生血清病，2 例出现轻度肝功能异常，1 例继发溶血，未发现因 p–ALG 应用后出现严重药物不良反应导致治疗失败的病例，对症治疗后均能控制，不良反应均能耐受。死亡患者中有 1 例治疗 2 个月后并发溶血，但因家庭矛盾及患者心理抵抗力弱自杀而亡，非本病直接导致的死亡。1 例患者 3 年后检测到 PNH 克隆，但无法明确为 SAA 自然病程转化还是治疗相关。无恶性克隆性疾病发生。

总之，国产 p–ALG 联合 CsA 治疗 SAA 长期效果肯定，不良反应能耐受，但花费低，可以作为经济状况不佳及不能行造血干细胞移植 SAA 患者的首选治疗。

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·临床报道·

猪抗人淋巴细胞免疫球蛋白联合环孢菌素 A 治疗重型再障的临床疗效

王方方，孙爱红，管俊*
（江苏省苏北人民医院血液科，扬州 225001）

【摘要】 目的 探讨使用国产猪抗人淋巴细胞免疫球蛋白(p-ALG)联合环孢菌素 A(CsA)治疗重型再生障碍性贫血(SAA)的疗效及观察不良反应。方法 应用 p-ALG(30 mg/kg/d，静脉滴注)联合 CsA(起始量为 5 mg/kg/d)治疗 SAA 8 例，辅以促造血、成分血输注、造血生长因子等支持治疗。检测治疗前后外周血象及骨髓象变化，以流式细胞仪测定治疗前后外周血 T 淋巴细胞亚群的改变情况，观察不良反应及其处理效果。结果 随访 8 例中，基本治愈 2 例，缓解 3 例，明显进步 1 例，早期死亡 2 例，总有效率 75%。治疗显效时患者的外周血 CD3+CD4+(Th)细胞比例升高，CD3+ CD8+(Ts)细胞比例降低，Th/Ts 比值升高，与治疗前比较，差异有统计学意义($P<0.01$)。结论 使用国产 p-ALG 联合 CsA 作为初诊 SAA 的首次治疗方案效果良好，治疗费用较马或兔抗胸腺细胞免疫球蛋白(ATG)联合 CsA 方案更经济。

【关键词】 猪抗人淋巴细胞免疫球蛋白；再生障碍性贫血；环孢素 A
【中图分类号】 R556.5 【文献标志码】 B

Clinical Efficacy of Pig Anti-Human Lymphocyte Immune Globulin Concomitant with Cyclosporine A on Severe Aplastic Anemia

*WANG Fang-fang, SUN Ai-hong, GUAN Jun** (*Department of Hematology, Subei People's Hospital, Yangzhou* 225001, *China*)

通讯作者：管俊，E-mail：guanjun-tt@163.com

综上所述，糖尿病患者发生肾脏损害时，如果糖尿病病程较短、糖尿病视网膜病变发生率较低、有血尿特别是肉眼血尿时，应高度怀疑患者合并有 NDRD，需及时进行肾活检以明确诊断，从而更好地指导患者治疗。

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【Abstract】 **Objective** To explore the clinical efficacy of pig anti-human lymphocyte immune globulin (p-ALG) concomitant with cyclosporine A (CsA) on severe aplastic anemia (SAA) and to observe their adverse responses. **Methods** Intravenous injections of 30 mg/kg/d p-ALG concomitant with 5 mg/kg/d CsA were used on 8 SAA patients. Support therapies such as injection of blood components and hematopoietic growth factor were supplemented. Peripheral hemogram and knochenbild myelogramm changes before and after treatment were detected, while T lymphocyte subgroups in peripheral blood before and after treatment were determined by flow cytometry. Adverse responses and the processing effect were observed. **Results** Of the 8 patients finished follow-ups, 2 were completely cured, 3 were remised, 1 was improved and 2 died early, with the total response rate being 75%. In patients with excellent efficacy, the CD3+CD4+(Th) cell ratio and Th/Ts ratio increased while CD3+ CD8+(Ts) cell ratio decreased, which had significant differences from treatment before ($P<0.05$). **Conclusion** p-ALG concomitant with CsA have favorable efficacy in the initial treatment of SAA, and cost less than horse or rabbit anti-thymocytes immune globulin concomitant with CsA.

【Key words】 Pig Anti-Human Lymphocyte Immune Globulin; Severe Aplastic Anemia; Cyclosporine A

再生障碍性贫血是一种骨髓造血功能衰竭症，主要表现为骨髓造血功能低下、全血细胞减少与不同程度的贫血、出血和感染征候群。重型再生障碍性贫血(severe aplastic anemia，SAA)是其严重表现，我国临床上分为急性重型再生障碍性贫血(SAA-I)和慢性重型再生障碍性贫血(SAA-II)[1]。SAA 的标准疗法为有人类白细胞抗原(HLA)相合的同胞供体行同种异体骨髓移植或造血干细胞移植，或联合抗人胸腺细胞免疫球蛋白(ATG/ALG)和环孢菌素 A(cyclosporin A，CsA)的免疫抑制治疗(immunosuppressive therapy，IST)。联合 IST 可作为无合适供者或不适合移植的 SAA 首选[1,2,8]。联合方案的强化免疫治疗可提高疗效，减轻大剂量单一药物的毒副作用[2]，既往多使用进口的 ATG。本文采用国产 p-ALG 联合 CsA 治疗 SAA，观察其疗效以及不良反应。

1 资料与方法

1.1 临床资料

选择我院血液科 2012 年 8 月～2013 年 12 月确诊并采用 p-ALG 联合 CsA 治疗的 SAA 患者共 8 例，其中男 4 例，女 4 例，年龄 13～57 岁，平均年龄 33.7 岁，随访时间 1～16 个月，中位随访时间 8 个月。8 例患者中初诊 SAA-I 7 例，SAA-II 1 例(面色苍白、牙龈出血 18 年，加重半年余)，所有患者入院前未接受任何 IST。确诊依据临床表现、骨髓穿刺和骨髓活检的结果，并排除引起全血细胞减少的其它疾病[1,6](见表 1)。

表 1 患者一般情况

病例号	性别	年龄/岁	体质量/kg	诊断	病程	临床表现	治疗场所
病例 1	男	13	52	SAA-I	1 月	面色苍白、牙龈出血	单人病房
病例 2	男	31	62	SAA-II	18 年	面色苍白、牙龈出血	净化层流仓
病例 3	女	33	51	SAA-I	1 月	皮肤瘀点、瘀斑、牙龈出血	净化层流仓
病例 4*	女	34	58	SAA-I	半月	发热、咽痛，牙龈出血	净化层流仓
病例 5	女	47	59	SAA-I	20 d	皮肤瘀点、瘀斑、牙龈出血	净化层流仓
病例 6	女	49	65	SAA-I	1 月	面色苍白、头昏乏力	单人病房
病例 7#	男	55	67	SAA-I	1 w	头痛、发热	净化层流仓
病例 8#	男	57	67	SAA-I	10 d	皮肤瘀点、瘀斑、牙龈出血	净化层流仓

* p-ALG+CsA 治疗后 1.5、3.5 个月输注脐带间充质干细胞 2 次(细胞数为 1×10^6/kg)；# 治疗后死亡患者

1.2 治疗方法

1.2.1 *联合治疗* 1) p-ALG，250 mg/支，武汉生物制品研究所产品，批号国药准字 S1083001。使用剂量为 30 mg/kg/d，稀释于 500 mL 生理盐水注射液中，静脉滴注。使用前行 ALG 皮试，皮试阴性者才可使用；若皮试阳性，取每支 1/10 量静脉滴注 1 h，观察不良反应，如无情况则常规治疗。用 ALG 前 0.5 h 肌注异丙嗪 25 mg，开始滴速为 5～10 滴/min，如 10 min 后无反应，再逐渐加速，维持 12 h。同时建立另一条静脉通路滴注甲强龙 1 mg/kg，同

步维持 12 h;1 w 后甲强龙逐渐减量,共用 1 月,根据有无血清病调整剂量。2)CsA,商品名:新赛斯平,50 mg/粒,杭州中美华东制药有限公司产品。使用剂量为 5 mg/kg/d,分 3 次口服。根据血药浓度(维持谷浓度 150～250 ng/mL)、肝肾功能调整药物用量,持续治疗 12 个月以上[1,2,4]。

1.2.2 *辅助治疗* 所有患者联合使用粒细胞集落刺激因子(G-CSF)、白介素 11(IL-11)和安雄。洁欣(300 μg/支,江苏吴中实业股份有限公司)300 μg/d、瑞白(100 μg /支,齐鲁制药厂)200 μg /d,均为皮下注射;IL-11(1.5 mg/支,齐鲁制药厂)3 mg/d,皮下注射。常规口服安雄(十一酸睾丸酮,40 mg/粒,南京欧加农制药厂)40 mg/tid。

1.2.3 *支持治疗* 8 例患者中 6 例在净化层流仓、2 例在普通单人病房进行治疗,每日进行口、眼、鼻、耳、皮肤、肛周和会阴部的护理。单人病房常规进行紫外线消毒,陪护人员穿隔离衣、戴口罩和帽子。治疗过程中应用复方新诺明(SMZ)、哌拉西林他唑巴坦或头孢唑肟、病毒唑(利巴韦林)或阿昔洛韦、伏立康唑或伊曲康唑预防抗感染治疗。

1.3 实验室检测

定期监测患者 CsA 血药浓度、肝肾功能、血常规、网织红细胞及骨髓象。在治疗前及治疗后 3、6 个月分别做 T 淋巴细胞亚群分析(BD 公司 cantoII 型行 FCM 检测)。

1.4 疗效判定

参考张之南血液病诊断及疗效标准(第 2 版)[6]。基本治愈:贫血和出血症状消失,血红蛋白(Hb)男性达 120 g/L、女性达 110 g/L,白细胞计数(WBC)达 4×10^9/L,血小板计数(BPC)达 100×10^9/L;缓解:贫血和出血症状消失,Hb 男性达 120 g/L、女性达 100 g/L,WBC 达 3.5×10^9/L,BPC 也有一定程度增加,随访 3 个月病情稳定或继续进步;明显进步:贫血和出血症状明显好转,不输血,Hb 较治疗前 1 个月内常见值增长 30 g/L 以上,并能维持 3 个月。判断以上 3 项疗效标准者,均应 3 个月内不输血。

1.5 统计学方法

采用 SPSS 18.0 软件对统计数据进行分析处理。计量资料以均数±标准差($\bar{x}\pm s$)表示,采用配对 t 检验,$P<0.05$ 为差异有统计学意义。

2 结果

2.1 临床疗效

8 例 SAA 患者在治疗后进行定期随访,在 3、6 个月时评估疗效,结果基本治愈 2 例,缓解 3 例,明显进步 1 例,治疗早期死亡 2 例(病例 7:55 岁,男性,完成 ALG 后 7 d,死因:肺部真菌感染;病例 8:57 岁,男性,完成 ALG 后 16 d,死因:粒细胞缺乏继发感染、电解质紊乱),总有效率为 75%。

2.2 治疗前后血液常规及骨髓检查

治疗后 3、6 个月的血常规检查显示 Hb、网织红细胞计数(Ret)、中性粒细胞(ANC)及血小板(PLT)等均有明显升高($P<0.01$)。在治疗后 3、6 个月进行骨髓检查显示,全部病例骨髓的有核细胞达到增生活跃-明显活跃,粒系、红系也有明显增生,易见巨核细胞(见表 2)。

表 2 p-ALG 联合 CsA 治疗 SAA 前后血常规检查($n=8$)

组别	Hb/(g/L)	Ret/%	ANC/($\times10^9$/L)	PLT/($\times10^9$/L)
治疗前	45.10±8.08	0.36±0.16	0.40±0.14	8.75± 6.45
治疗后 3 个月	80.20±8.79	1.77±0.23	1.86±0.61	46.50±10.37
t 值	15.28	33.74	10.26	9.89
P 值	0.01	0.01	0.01	0.01

2.3 T 淋巴细胞亚群在治疗前后的变化

与治疗前比较,患者治疗后的 CD3+CD4+(Th)细胞比例明显升高,CD3+CD8+(Ts)细胞比例降低,其比值显著升高,差异有统计学意义($P<0.01$)(见表 3)。

表 3 p-ALG 联合 CsA 治疗 SAA 前后 T 淋巴细胞亚群变化($n=8$)

组别	CD3+/%	Th/%	Ts/%	Th/Ts
治疗前	74.39±5.48	25.20±3.16	39.40±4.1	0.62±0.09
治疗后 3 个月	77.20±6.54	40.70±4.23	24.76±3.01	1.57±0.27
t 值	1.61	9.76	15.36	16.70
P 值	0.01	0.01	0.01	0.01

2.4 药物不良反应

所有患者中,下肢皮疹 1 例,血清胆红素、血糖、血肌酐升高 2 例,浮肿 3 例,谷草转氨酶(AST)、谷丙转氨酶(ALT)升高 5 例,发热 5 例(肺炎、泌尿道感染各 1 例),分别经抗过敏、保肝、抗感染及对症治疗后缓解、恢复。

3 讨论

目前认为 T 淋巴细胞功能亢进在原发性获得性再障发病机制中占重要地位,再障是 T 淋巴细胞介导的以造血系统为靶器官的自身免疫性疾病。近年多项研究[1-7]表明,SAA 的患者体内存在 CD4+/

CD8＋比值降低、Th1 细胞功能亢进等 T 淋巴细胞亚群失衡现象，提示 SAA 存在 T 淋巴细胞功能异常[9]。因此，抑制 CD8＋细胞及其功能成为 SAA 治疗的靶点。p-ALG 为武汉生物研究所独家生产，是通过用人胸腺细胞免疫猪而获得的抗人 T 淋巴细胞的特异性抗体，是一种选择性作用于 T 淋巴细胞的免疫抑制剂，其作用机制不只是清除血液及外周组织中的 T 细胞，还通过补体依赖的细胞溶解作用，促进 T 细胞激活及凋亡，影响树突状细胞（DC），调节介导白细胞-内皮细胞相互作用的细胞表面分子等，起到了多方面的免疫调节作用，有效率可达 50％～70％。

研究[10]表明，ATG/ALG 与 CsA 合用效果明显优于其中任何单药治疗。既往 IST 治疗 SAA 多用进口 ATG，而国产 p-ALG 联合 CsA 治疗 SAA 的临床资料不多，本研究 8 例患者均采用国产 p-ALG 联合 CsA 作为初治方案，并根据患者血药浓度及自身耐受情况调整 CsA 用量；治疗期间予以成分输血、G-CSF、IL-11 及抗感染等辅助支持治疗措施，基本治愈 2 例，缓解 3 例，明显进步 1 例，总有效率为 75％，与国内外应用马或兔 ATG 治疗 SAA 的疗效相似[10-12]。本研究结果显示，治疗后 3 个月患者血象、Ret、骨髓象明显改善，T 淋巴细胞亚群比值显著升高，与治疗前比较，差异有统计学意义（$P<0.01$）。本研究 2 例完全脱离输血，4 例输血时间明显延长，说明国产 p-ALG 联合 CsA 治疗 SAA 患者有较好疗效。

本研究中，患者对 p-ALG 耐受性良好，未出现严重的血清病反应，部分患者出现下肢皮疹、肝肾功能损害、浮肿等，均经过治疗后获得恢复。在治疗过程中，8 例 SAA 患者有 5 例发生不同程度的感染，其中病例 7、8 号两例患者死亡（重症感染）。这两例早期死亡的患者年龄均超过 55 岁，可认为患者年龄可能为影响疗效因素之一[2,3]。本研究还发现，普通单人病房接受治疗的两例患者（病例 1、6）在严格的保护隔离状态下，未发生严重感染，总治疗费用低于净化层流仓患者，说明国产 p-ALG 也可在普通单人病房使用，从而降低住院费用。此外，p-ALG 价格经济，患者住院 1 个月的总花费仅为马或兔 ATG 的 1/2，明显减少了医疗费用。

综上所述，本研究使用国产 p-ALG 联合 CsA 作为初诊 SAA 的治疗方案，效果良好，副反应小，建议选择患者年龄在 50 岁以下为宜。猪来源的免疫球蛋白相对于兔和马更便于获得，价格便宜，为效价比高的 IST 治疗主要药物之一，值得推广。本研究虽然病例数偏少，但也初步证明其良好的效果。

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(8.2 ± 1.6)IU/L，两组数据比较差异均有统计学意义 (P<0.05)。

3　讨论

多项研究显示，50% 以上的急性间质性肾炎都是由氟喹诺酮类药物引发的，患者常常伴有血尿症状，可能是由于药物结晶后，对患者的尿路黏膜造成了损伤。随着年龄的增加，机体功能逐渐衰弱，多数人群常常使用氟喹诺酮类药物，该药物具有较强的毒副作用，对患者造成的创伤显而易见，充分体现了积累效应，随着服用时间的增长，对肾脏造成的损害越严重。使患者长期处于肾损害状态，致使病情逐步恶化，导致肾功能衰竭，甚至危及患者性命，因此早期诊断和及时治疗，可以有效改善患者的预后情况[2-4]。现阶段，在诊断由氟喹诺酮类药物引发的急性间质性肾炎时，最主要的诊断依据就是血清检测、尿液检测和血液检测。在本次研究中，对所有研究对象均进行血清检测、尿液检测和血液检测，结果发现，观察组患者血清检查结果、尿液检测结果及血液检测结果中的各项含量水平明显高于健康人群，完全符合肾损害患者的临床表现。

综上所述，由氟喹诺酮类药物所引发的急性间质性肾炎，缺乏特异性，在对患者给药时，要对肾功能、尿常规、血常规进行定期检查，在急性间质性肾炎的早期诊断中，可以作为有效指标。

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猪抗人淋巴细胞球蛋白联合环孢素治疗重型再生障碍性贫血的疗效及安全性分析

陈昌茜　黄世华

【摘要】 目的　分析和探讨猪抗人淋巴细胞球蛋白联合环孢素治疗重型再生障碍性贫血的临床效果与安全性。**方法**　30 例重型再生障碍性贫血患者随机分为观察组和对照组，各 15 例。观察组采用猪抗人淋巴细胞球蛋白联合环孢素治疗，对照组患者采用环孢素治疗，比较两组患者临床治疗效果与不良反应发生情况。**结果**　观察组治疗有效率明显高于对照组，但同时不良反应发生率高于对照组，差异均具有统计学意义 (P<0.05)。**结论**　在重型再生障碍性贫血治疗中，猪抗人淋巴细胞球蛋白联合环孢素治疗效果显著，但不良反应较多，在使用时应注意并发症及不良反应的防治。

【关键词】 猪抗人淋巴细胞球蛋白；环孢素；重型再生障碍性贫血
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重型再生障碍性贫血是一种获得性骨髓造血功能衰竭，临床表现为严重贫血、感染及出血等[1]，预后凶险。针对不宜行造血干细胞移植患者，采用免疫抑制治疗，联合采用免疫球蛋白与环孢素，能明显降低各种免疫抑制剂用量，提高患者耐受力，减少药物毒副作用，有效率为 40%~70%。猪抗人淋巴细胞球蛋白因费用低、疗效好在国内临床治疗中广泛应用。本文选取本院 46 例重型再生障碍性贫血患者为对象，分析猪抗人淋巴细胞球蛋白联合环孢素治疗效果与不良反应。现报告如下。

1　资料与方法

1.1　一般资料　选取本院 2010 年 1 月 ~2015 年 1 月收治的 30 例重型再生障碍性贫血患者作为研究对象，男 19 例，女 11 例，年龄 20~70 岁，平均年龄 (38.2 ± 8.3) 岁，病程 15 d~8 个月，将其随机分为观察组和对照组，各 15 例。全部病例均符合重型再生障碍性贫血临床诊断标准[2]，治疗前肝、肾功能正常，排除骨髓增生异常综合征、阵发性睡眠性血红蛋白尿、范可尼贫血等，且发病前无病毒感染史。两组患者一般资料比较，差异无统计学意义 (P>0.05)，具有可比性。

1.2　方法　对照组患者环孢素治疗，首次 3~5 mg/(kg · d)，分 2 次口服，明显起效后缓慢减小至 0.5~2 mg/d，2 年为 1 个疗程，环孢素血药浓度控制在 200~400 ng/ml，同时服用护肝药物。观察组患者在此基础上联合猪抗人淋巴细胞球蛋白治疗，30 mg/(kg · d)，连用 5 d，同时予以甲泼尼龙 1~2 mg/(kg · d) 与猪抗人淋巴细胞球蛋白同步静脉滴注，猪抗人淋巴细胞球蛋白结束后，改为泼尼松 1 mg/(kg · d) 口服，并逐渐减量至 28 d 停用。

1.3　疗效评定标准[3]　治愈：临床症状消失，白细胞达到 4.0×10^9/L，血小板达 100×10^9/L，血红蛋白女 110 g/L、男 120 g/L 以上；缓解：临床症状消失，白细胞达到 3.5×10^9/L，血小板一定程度增加，血红蛋白女 110 g/L、男 120 g/L 以上；无效：临床症状及血象无明显变化。治疗有效率 = 治愈率 + 缓解率。

1.4　统计学方法　采用 SPSS17.0 统计学软件对数据进行统计分析。计量资料以均数 ± 标准差 ($\bar{x}\pm s$) 表示，采用 t 检验；计数资料以率 (%) 表示，采用 χ^2 检验。P<0.05 表示差异具有统计学意义。

2　结果

2.1　两组患者疗效比较　观察组患者治愈 9 例 (60.00%)，缓解 5 例 (33.33%)，无效 1 例 (6.67%)，治疗有效率为 93.33%，对照组患者治愈 5 例 (33.33%)，缓解 4 例 (26.67%)，无效 6 例 (40.00%)，治疗有效率为 60.00%，观察组治疗有效率高于对照

作者单位：644000　四川省宜宾第二人民医院血液科

组，差异有统计学意义（$P<0.05$）。

2.2　两组患者不良反应比较　对照组患者牙龈增生1例、手颤1例、继发性糖尿病1例，肝功损害1例，不良反应发生率为26.7%，给予环孢素减量或停药治疗，并加强保肝对症后均好转；观察组患者继发性糖尿病1例，发热1例、心悸1例、手颤1例、肝功损害2例、血清病反应4例，不良反应发生率为66.7%，给予环孢素减量及激素加量及加强保肝对症治疗后好转；观察组患者不良反应发生率高于对照组（$P<0.05$）。

3　讨论

再生障碍性贫血是一种免疫介导骨髓衰减性疾病，与T淋巴细胞产生可溶性抑制因子攻击造血干细胞导致的免疫介导性损伤密切相关。临床多采用骨髓移植治疗，但仅有少数患者能配型成功，多数患者需强化免疫抑制治疗。免疫治疗方案（IST）是非移植重型再生障碍性贫血患者的标准方案[4]，其临床效果十分显著。常用的进口马或兔抗淋巴细胞免疫球蛋白价格昂贵，患者经济压力较大，对治疗产生一定的影响。

本研究采用国内猪抗人淋巴细胞球蛋白，其价格相对低廉，联合环孢素治疗重型再生障碍性贫血，不仅提高了治疗效果，同时降低了患者医疗经济负担。

本组研究结果表明，观察组患者治疗有效率明显高于对照组，结果提示猪抗人淋巴细胞球蛋白与马、兔抗人淋巴细胞球蛋白疗效相当，与环孢素联合治疗重型再生障碍性贫血效果显著。此外，观察组不良反应发生率高于对照组，即联合用药不良反应多，减少环孢素剂量和对症治疗后均好转，可见猪抗人淋巴细胞球蛋白耐受性与安全性较好。

综上所述，在临床重型再生障碍性贫血治疗中，采用猪抗人淋巴细胞球蛋白联合环孢素治疗效果显著，但联合用药不良反应多，需进一步探究不良反应及并发症的防治措施，从而改善临床症状，提高临床治疗效果。

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尿道内切开联合曲安奈德治疗尿道狭窄效果分析

查仕方　陈克俭　唐巍

【摘要】 **目的**　分析尿道切开联合曲安奈德应用于尿道狭窄的效果。**方法**　68例男性尿道狭窄患者随机分成观察组和对照组，各34例。观察组给予尿道内切开术联合曲安奈德治疗，对照组给予尿道内切开术治疗，对比治疗后两组的治疗效果、最大尿流量、狭窄段内径和复发情况。**结果**　观察组的治疗总有效率为94.12%，最大尿流量为（20.82±1.35）ml/s，狭窄段内径为（4.66±0.35）mm，复发率为5.88%，均优于对照组（$P<0.05$）。**结论**　尿道内切开联合曲安奈德能有效提高治疗总有效率，加大尿流量，扩大狭窄段内径，减少复发率，值得推广。

【关键词】 尿道切开；联合；曲安奈德；尿道狭窄

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尿道狭窄是一种常见的内分泌疾病，男性的发病率多于女性，且发病原因以炎症性、外伤性尿道狭窄以及医源性损伤为主。其早期症状为排尿不畅、尿流变细、排尿时常中断等，病情发展时可出现尿潴留、尿失禁等症状。如不及时进行治疗，严重者可发展成肾积水、肾萎缩、肾功能不全等疾病[1]。临床上大多是给予患者手术治疗，本院为探究尿道切开联合曲安奈德应用于尿道狭窄的临床疗效，特采用两组不同的方法对患者进行治疗，并对比两组患者的治疗效果。现报告如下。

1　资料与方法

1.1　一般资料　选取2013年4月~2014年4月本院收治的68例男性尿道狭窄患者作为研究对象，随机分为观察组和对照组，各34例。观察组年龄30~60岁，平均年龄（44.26±4.68）岁，14例炎症性，20例外伤性，22例前尿道狭窄，12例后尿道狭窄。对照组年龄32~60岁，平均年龄（45.52±2.73）岁，13例炎症性，21例外伤性，24例前尿道狭窄，10例后尿道狭窄。两组患者在年龄、狭窄原因以及狭窄部位疾病类型方面比较，差异无统计学意义（$P>0.05$），具有可比性。

1.2　诊断标准　①均有排尿困难、排尿延缓、尿流细而无力、不成线等临床症状；②均有尿道损伤史以及炎症史，但无尿瘘、腹股沟疝、肛门直肠脱垂等并发症；③尿道长度≤1.5 cm，内径≤1.35 cm；④均无使用皮质类固醇激素药物。

1.3　治疗方法　所有患者均在手术前接受常规准备，如测量体温、脉搏、呼吸、血压、心率、血型等，并进行药物过敏测试、尿细菌培养以及常规抗感染治疗。对照组采用尿道内切开术治疗，具体如下：①采用硬膜外麻醉，体位取截石位。②将尿道镜从尿道外口插至患者狭窄部位顶端，接近顶端时要前后移动，以明确狭窄长度和瘢痕范围。③把尿道镜

作者单位：516006　广东省惠州市中信惠州医院

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猪抗人淋巴细胞球蛋白联合环孢素治疗重型再生障碍性贫血

钟淑萍, 曾林涓, 李学刚, 谢 锋, 庞文正, 何志国, 侯丽君, 徐景勃
(中山大学附属第五医院, 广东 珠海 519000)

[关键词] 贫血, 再生障碍性; 抗淋巴细胞血清; 环孢素; 猪抗人淋巴细胞球蛋白

[摘要] 目的 评价猪抗人淋巴细胞球蛋白联合环孢素治疗重型再生障碍性贫血的疗效及安全性, 并分析影响疗效的相关因素。方法 回顾性分析 2002 年 1 月至 2012 年 3 月本院 32 例应用抗人 T 细胞猪免疫球蛋白 (第 1 ~ 5 日, 20 ~ 30 $mg\cdot kg^{-1}\cdot d^{-1}$) 联合环孢素 (起始量 3 ~ 5 $mg\cdot kg^{-1}\cdot d^{-1}$) 治疗的重型再生障碍性贫血患者资料。评价治疗 3、6、12 个月末的临床疗效及不良反应发生情况, 并分析患者性别, 年龄, 治疗前中性粒细胞、淋巴细胞、网织红细胞计数与疗效的关系。结果 治疗 12 个月末, 32 例患者中 14 例基本治愈 (44%)、7 例缓解 (22%), 3 例明显进步 (9%), 8 例无效 (25%), 总有效率为 75%。主要不良反应为发热 (56%)、皮疹 (44%)、血清病 (19%)、肝功能损害 (25%)。单因素分析表明诊断至治疗的时间 < 30 d、治疗前中性粒细胞 $\geqslant 0.2\times10^9\cdot L^{-1}$ 及治疗前无合并感染的患者有效率高于诊断至治疗的时间 $\geqslant$ 30 d、治疗前中性粒细胞 $< 0.2\times10^9\cdot L^{-1}$ 及治疗前合并感染的患者 ($P < 0.05$), 患者性别、年龄、治疗前网织红细胞计数对疗效影响不大; Logistic 回归分析表明诊断至治疗的时间是影响疗效的唯一因素 ($P < 0.05$)。结论 猪抗人淋巴细胞免疫球蛋白联合环孢素治疗重型再生障碍性贫血疗效理想, 不良反应可耐受。诊断至治疗的时间、治疗前中性粒细胞水平及是否合并感染是影响疗效的主要因素。

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[作者简介] 钟淑萍, 女, 主治医师, 硕士研究生, 主要从事血液病的诊治, E-mail: peakzhong@21cn.com; 徐景勃, 男, 副主任医师, 硕士研究生, 主要从事血液病的诊治, E-mail: xudongguang@126.com
[责任作者] 徐景勃

Porcine antilymphocyte globulin plus cysclosporine in treatment of severe aplastic anemia

ZHONG Shu-ping, ZENG Lin-juan, LI Xue-gang, XIE Feng, PANG Wen-zheng, HE Zhi-guo, HOU Li-jun, XU Jing-bo

(*The Fifth Affiliated Hospital of SUN YAT-SEN University, Zhuhai GUANGDONG 519000, China*)

[**KEY WORDS**] anemia, aplastic; antilymphocyte serum; cysclosporine; porcine antilymphocyte globulin

[**ABSTRACT**] AIM To investigate the efficacy and safety of porcine antilymphocyte globulin (pALG) plus cysclosporine therapy for severe aplastic anemia (SAA), and to analyze the related factors affect the efficacy. METHODS The clinical data of 32 patients with SAA from January 2002 to March 2012 in our hospital were retrospectively evaluated. The patients were treated with a combination of anti-human T lymphocyte porcine immunoglobulin (20 – 30 $mg \cdot kg^{-1} \cdot d^{-1}$, d1–d5) and cysclosporine (3 – 5 $mg \cdot kg^{-1} \cdot d^{-1}$ initially). The clinical efficacy was evaluated at the end of 3, 6 and 12 months after the treatment. The related factors affect the curative effects were also analyzed. RESULTS At the end of 12 months after the treatment, the overall response was 75% (44% complete, 22% partial response, 9% improved). The main adverse reactions included fever (56%), skin rashes (44%), serum sickness (19%), impaired liver function (25%). Single factor analysis revealed that the effective rate of patients with interval between diagnosis and treatment < 30 days, the level of neutrophils ≥ $0.2 \times 10^9 \cdot L^{-1}$ and absence of infection before immunosuppressive therapy were higher than those of patients with related with interval between diagnosis and treatment ≥ 30 days, the level of neutrophils < $0.2 \times 10^9 \cdot L^{-1}$ and infection before immunosuppressive therapy ($P < 0.05$). Sex, age, and the reticulocyte cell counts before the treatment had few effects on the therapy response. Logistic regression analysis showed that interval between diagnosis and treatment was the only factor affected the therapy response ($P < 0.05$). CONCLUSION PALG plus cyclosporine is an ideal therapy for SAA and adverse reactions could be tolerated. The interval between diagnosis and treatment, the level of neutrophils and pre-existing infection influenced the efficacy.

重型再生障碍性贫血（简称重型再障）**以骨髓造血功能衰竭和全血细胞减少为特征，临床上表现为严重贫血、感染和出血**[1]。**马抗胸腺细胞球蛋白**（horse antithymocyte globulin，hATG）**联合环孢素**（cyclosporine，CsA）**的免疫抑制治疗**（IST）**是非移植重型再障患者的标准方案**[2]。**猪抗人淋巴细胞球蛋白**（porcine antilymphocyte globulin，pALG）**已在国内临床广泛应用多年，但相关报道较少。本研究回顾性分析本院使用国产** pALG **联合** CsA **治疗初治重型再障患者的资料，评价其疗效和不良反应，并分析影响疗效的因素，现报道如下。**

资料和方法

病例选择 **本院** 2002 **年** 1 **月至** 2012 **年** 3 **月住院使用** pALG + CsA **治疗的初治重型再障患者共** 32 **例，诊断标准参见文献**[3]。**所有患者均无其他可引起三系减少的疾病，如阵发性睡眠性血红蛋白尿、骨髓增生异常综合征、自身抗体介导的全血细胞减少、急性造血功能停滞、骨髓纤维化、急性白血病、恶性组织细胞病等。**

32 **例患者中男性** 22 **例，女性** 10 **例，中位年龄** 37 **岁**（12 ~ 56 **岁**），**中性粒细胞计数** $0.232 \times 10^9 \cdot L^{-1}$ [$(0.02 \sim 0.96) \times 10^9 \cdot L^{-1}$]，**血红蛋白** 61 $g \cdot L^{-1}$（43 ~ 115 $g \cdot L^{-1}$），**网织红细胞计数** $11 \times 10^9 \cdot L^{-1}$ [$(1 \sim 21) \times 10^9 \cdot L^{-1}$]，**血小板计数** $5.3 \times 10^9 \cdot L^{-1}$ [$(2 \sim 23) \times 10^9 \cdot L^{-1}$]，**淋巴细胞计数** $1.1 \times 10^9 \cdot L^{-1}$ [$(0.73 \sim 1.97) \times 10^9 \cdot L^{-1}$]。**所有患者均未检测到异常染色体核型。**

治疗方案 **治疗用药包括** pALG、CsA、**甲泼尼松龙和重组人粒细胞刺激因子。抗人** T **细胞猪免疫球蛋白**（anti-human T lymphocyte porcine

immunoglobulin，武汉生物制品研究所生产，规格：每瓶 5 mL，含蛋白质 0.25 g；批准文号：国药准字 S10830001)：第 1 ~ 5 日，20 ~ 30 mg·kg^{-1}·d^{-1}。CsA 胶囊（杭州中美华东制药有限公司，批准文号：国药准字 H10960121)：起始量 3 ~ 5 mg·kg^{-1}·d^{-1}，根据 CsA 血药浓度及患者肝、肾功能调整用量；维持治疗期间每月随访 1 次，根据血常规结果逐渐减量，至少维持治疗 2 年以上。注射用甲泼尼龙（methylprednisolone，辉瑞制药比利时公司)：第 1 ~ 5 日，40 ~ 80 mg·d^{-1}，根据有无血清病调整剂量，30 d 内减停。重组人粒细胞刺激因子注射液（recombinant human granulocyte colony stimulating factor injection，杭州九源基因工程有限公司）450 μg·d^{-1}，直至中性粒细胞计数大于 0.5 × 10^9·L^{-1}。治疗前合并感染患者均给予抗感染治疗，感染症状好转、体温正常 3 d 以上方给予免疫抑制治疗。

观察指标 评价治疗 3、6、12 个月末临床疗效，疗效标准参照《血液病诊断及疗效标准》，分为基本治愈、缓解、明显进步和无效，基本治愈、缓解和明显进步合计为有效，计算总有效率。统计药物不良反应及合并感染情况。治疗 12 个月分析患者性别，年龄，治疗前中性粒细胞、淋巴细胞、网织红细胞计数与疗效的关系。

统计学方法 采用 SPSS13.0 软件进行统计学分析。数据均用中位数表示，单因素分析采用 Fisher 确切概率法，多因素分析采用 logistic 回归。$P < 0.05$ 为差异有显著意义。

结 果

临床疗效 诊断至治疗时间为 56 d（3 ~ 72 d)，治疗至起效时间为 95 d（33 ~ 125 d)。治疗 3、6、12 个月末临床疗效评价结果见表 1。

表 1 不同治疗时间段的疗效评价 n = 32，例（%）

时间	基本治愈	缓解	明显进步	无效	总有效
3 个月末	4（12）	8（25）	8（25）	12（38）	20（62）
6 个月末	13（41）	8（25）	3（9）	8（25）	24（75）
12 个月末	14（44）	6（19）	4（12）	8（25）	24（75）

不良反应 接受治疗后，18 例（56%）患者在静滴 pALG 过程中和治疗后 2 周内出现发热，给予甲强龙治疗后体温控制正常。14 例（44%）出现不同程度的皮疹，6 例（19%）出现血清病，8 例（25%）出现丙氨酸转氨酶和（或）天冬氨酸转氨酶升高，未出现肌酐升高、过敏性休克及因 pALG 并发症而死亡的病例。

感染情况 治疗前有 13 例患者合并不同程度感染，主要感染部位依次是咽（5 例)、肺（4 例)、皮肤（3 例）和尿路（2 例)。免疫抑制治疗后，32 例患者中有 19 例（60%）并发感染，其中 14 例发生于治疗后的 3 ~ 4 周严重粒缺期，经广谱抗生素治疗后有 4 例继发肺部真菌感染，但无死亡病例。

影响疗效的因素分析 单因素分析结果表明，患者的性别、年龄、治疗前网织红细胞计数对疗效影响不大（$P > 0.05$)，而诊断至治疗时间 < 30 d、治疗前中性粒细胞 ≥ 0.2 × 10^9·L^{-1} 和治疗前未合并感染的患者有效率高于诊断至治疗的时间 ≥ 30 d、治疗前中性粒细胞 < 0.2 × 10^9·L^{-1} 及治疗合并感染的患者（$P < 0.05$)，见表 2。进一步用 logistic 回归分析方法筛选影响疗效的因素，结果表明诊断至治疗的时间是影响疗效的唯一因素（$P < 0.05$)，见表 3。

表 2 影响患者疗效的因素分析 例

影响因素		无效（n = 8)	有效（n = 24）	P 值
性别	男	6	16	0.665
	女	2	8	
年龄	> 40 岁	5	13	0.685
	≤ 40 岁	3	11	
诊断至治疗时间	< 30 d	2	20	0.002
	≥ 30 d	6	4	
治疗前合并感染	是	6	7	0.030
	否	2	17	
治疗前指标				
中性粒细胞	< 0.2 × 10^9·L^{-1}	5	5	0.024
	≥ 0.2 × 10^9·L^{-1}	3	19	
网织红细胞	< 10 × 10^9·L^{-1}	5	9	0.224
	≥ 10 × 10^9·L^{-1}	3	15	
淋巴细胞	< 1 × 10^9·L^{-1}	2	11	0.420
	≥ 1 × 10^9·L^{-1}	6	13	

各指标不同级别间比较，采用 Fisher 确切概率法

表 3 影响疗效的多因素 logistic 回归分析结果

影响因素	b	SE	Wald	df	P 值	exp（b）
诊断至治疗的时间	−3.207	1.244	6.642	1	0.010	0.040
治疗前是否合并感染	−2.408	1.265	3.625	1	0.057	0.090
Constant	3.845	1.315	8.548	1	0.003	46.776

讨 论

再生障碍性贫血是免疫介导的骨髓衰竭性疾病，与自身反应性 T 淋巴细胞产生可溶性抑制因子攻击造血干细胞导致的免疫介导性损伤有关[5]，因此 IST 成为再障患者的一线治疗方案，其疗效亦得到临床证实。重型再障的传统治疗方案为 hATG

联合 CsA，有效率 60% ~ 80%，5 年生存率 75% ~ 80%[6]，随着支持治疗的进步，目前生存率有进一步提高。兔抗胸腺细胞球蛋白（rabbit antithymocyte globulin，rATG）主要推荐用于 hATG 无效或复发的患者[7, 8]。进口的 hATG 和 rATG 治疗费用均昂贵，使国内很多患者错失治疗良机。国产 pALG 价格适中，国内很多医院选择这种药物治疗重型再障。本研究分析数据表明，国产 pALG 联合 CsA 治疗重型再障的 3 个月有效率为 62%，6 个月和 1 年有效率可达 75%，基本与进口 ATG 疗效相当。虽然 pALG 联合 CsA 治疗重型再障多数在 3 个月内见效，但是大部分患者在接受治疗后 6 个月时才达到最佳效果，6 个月至 1 年期间疗效未见进一步改善。2009 年英国血液病学会的指南提出，单疗程 IST 治疗无效或复发患者推荐第二次使用 ATG，两次间隔不能短于 3 个月[6]，而 2010 年再生障碍性贫血诊断治疗专家共识中则提出两次 IST 应间隔 6 个月[3]。本研究结果提示中国的再障诊断与治疗专家共识的建议对临床治疗决策更有指导意义。pALG 的不良反应主要是发热，本研究中一半以上的患者出现发热，给予甲强龙治疗后体温控制正常。肝功能损害患者给予护肝药物治疗后肝功能正常，未出现严重肝功能损害。因此我们认为，国产 pALG 联合 CsA 治疗重型再障疗效满意，不良反应可耐受，且价格适中，值得临床推荐应用。

如何预测和提高 IST 的疗效一直是临床研究的热点之一，因为它可为 IST 后进一步治疗方案的制订提供更多的信息，从而减少治疗的被动性和盲目性，但是至今为止尚无明确和可靠的预测指标。SCHEINBERG 等[9]回顾性分析了 316 例接受 hATG 联合 CsA 治疗的患者发现，年轻患者、治疗前高网织红细胞绝对数和高淋巴细胞计数对 6 个月治疗高反应率有预测作用。另一项研究表明，治疗前的中性粒细胞计数是唯一可预测治疗反应率的因素，而高网织红细胞计数是长期生存率的预测指标[10]。但是这些研究结果均未得到临床医生的广泛认同，大多数临床医生认为再障患者明确诊断后越早治疗疗效越好，在干细胞大量凋亡、骨髓造血组织完全耗竭之前进行 IST 治疗，一方面可阻断免疫介导的骨髓衰竭继续加重，另一方面可减少粒缺继发感染的死亡率。本研究结果表明，诊断至治疗的时间 < 30 d、治疗前中性粒细胞 ≥ $0.2 \times 10^9 \cdot L^{-1}$ 及治疗前无合并感染的患者疗效较好，但进一步用 logistic 回归分析筛选影响因素，发现诊断至治疗的时间是唯一影响疗效的因素。由于本研究病例数量较少，logistic 回归分析的结果偏于保守，结论尚需要大样本进行证实。

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·论著·

ATG/ALG联合环孢素A与环孢素A联合雄激素一线治疗输血依赖非重型再生障碍性贫血的疗效比较:单中心回顾性研究

宋琳 彭广新 武志洁 张莉 井丽萍 周康 李洋 李园
叶蕾 李建平 樊慧慧 赵馨 杨文睿 杨洋 张凤奎

【摘要】 **目的** 比较抗人胸腺/淋巴细胞球蛋白(ATG/ALG)联合环孢素A(CsA)与CsA联合雄激素一线治疗输血依赖非重型再生障碍性贫血(TD-NSAA)疗效。**方法** 回顾性分析2007年8月至2014年9月125例TD-NSAA患者临床资料,比较一线采用ATG/ALG联合CsA与CsA联合雄激素治疗的血液学反应及生存情况。**结果** 125例TD-NSAA患者中,男70例,女55例,男女比为1.27∶1;中位年龄27(6~66)岁。其中48例一线接受ATG/ALG联合CsA治疗,77例一线接受CsA联合雄激素治疗,两组早期死亡率分别为2.1%(1/48)及0(0/77)(*P*=0.384)。ATG/ALG联合CsA组患者治疗后3个月总体血液学反应率(70.8%对45.5%,*P*=0.006)和良好血液学反应率(27.1%对10.4%,*P*=0.015)均高于CsA联合雄激素组;两组治疗后6个月总体血液学反应率(75.0%对55.8%,*P*=0.031)与良好血液学反应率(41.7%对22.1%,*P*=0.020)差异亦有统计学意义,治疗后6个月ATG/ALG联合CsA组脱离血制品输注依赖的中位时间为36.5(0~149)d,明显短于CsA联合雄激素组的98(14~180)d(*P*<0.001)。ATG/ALG联合CsA组与CsA联合雄激素组患者3年总生存率(97.9%对100.0%,*P*=0.227)和无事件生存率(71.2%对59.5%,*P*=0.227)差异无统计学意义。**结论** 一线采用CsA联合雄激素治疗TD-NSAA血液学反应率和血液学反应质量均不及ATG/ALG联合CsA,两组患者短期生存率相同,应优选ATG/ALG联合CsA方案治疗TD-NSAA。

【关键词】 输血依赖; 贫血,再生障碍性; 免疫抑制法
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Treatment of transfusion-dependent nonsevere aplastic anemia with cyclosporine A plus ATG/ALG versus cyclosporine A plus androgens: a retrospective single center study *Song Lin, Peng Guangxin, Wu Zhijie, Zhang Li, Jing Liping, Zhou Kang, Li Yang, Li Yuan, Ye Lei, Li Jianping, Fan Huihui, Zhao Xin, Yang Wenrui, Yang Yang, Zhang Fengkui. Institute of Hematology and Blood Disease Hospital, CAMS & PUMC, Tianjin 300020, China*
Corresponding author: Zhang Fengkui, Email:zhfk@hotmail.com

【**Abstract**】 **Objective** To determine whether cyclosporine A (CsA) plus androgens was as effective as the current standard immunosuppressive therapy (IST) for transfusion-dependent nonsevere aplastic anemia (TD-NSAA). **Methods** The records of 125 consecutive TD-NSAA patients who were treated between Aug. 2007 and Sept. 2014, with either CsA plus androgen or ALG/ATG plus CsA regimen were reviewed. The 3-month and 6-month hematologic responses and survival were evaluated. **Results** There were 125 TD-NSAA patients (70 were male and 55 female, 1.25∶1). Median age was 27 (6-66) years. There was no significant difference in early mortality between 48 treated by ATG/ALG plus CsA and 77 by CsA plus androgen patients (1/48 *vs* 0/77, *P*=0.384). Both the total hematologic response and the better hematological response rates at 3-month (70.8% *vs* 45.5%, *P*=0.006 and 27.1% *vs* 10.4%, *P*=0.015, respectively) and 6-month (75.0% *vs* 55.8%, *P*=0.031 and 41.7% *vs* 22.1% *P* = 0.020, respectively) after

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作者单位:300020 天津,中国医学科学院、北京协和医学院血液学研究所、血液病医院
通信作者:张凤奎,Email:zhfk@hotmail.com

treatment were much higher in the standard IST group than that in CsA plus androgen group. The median time to transfusion independent of 36.5(0-149) days in the standard IST group was significantly shorter than 98(14-180) days in CsA plus androgen group (P<0.001). Survival was comparable between the two groups (97.9% *vs* 100.0%, P=0.227). It was superior (71.2% *vs* 59.5%) but not significantly (P=0.227) in event-free survival in standard IST group. **Conclusions** CsA plus androgen was inferior to the standard IST of ATG/ALG and CsA regimen in treating TD-NSAA in terms of the hematologic response and the quality of response, despite of comparable short-term survival.

【**Key words**】 Transfusion dependence; Anemia, aplastic; Immunosuppression

Fund program: Clinical Medical Professional Degree Postgraduate Clinical Research Ability Cultivation Model (PUMCGS-2012009)

再生障碍性贫血(AA)是一种少见的骨髓造血衰竭综合征。骨髓造血干/祖细胞池在异常活化的自身细胞毒性T淋巴细胞及其释放的细胞因子作用下进行性萎缩,致外周血细胞不同程度减少,感染、出血风险升高,严重者危及生命。对于重型和极重型AA(SAA/VSAA)患者,明确诊断后须尽早行异基因造血干细胞移植或免疫抑制治疗(IST)已取得共识;但对非重型AA(NSAA)开始治疗的时机及治疗方案的选择尚未达成一致,尤其输血依赖NSAA(TD-NSAA)不仅缺乏明确定义,而且有关TD-NSAA患者接受IST疗效的报告也较少。迄今仅两项前瞻性研究报告了TD-NSAA患者一线接受IST的结果[1-2]。本研究中,我们回顾性分析125例TD-NSAA患者临床资料,比较抗人胸腺/淋巴细胞球蛋白(ATG/ALG)联合环孢素A(CsA)的标准IST方案与CsA联合雄激素治疗的疗效及生存情况,现报道如下。

病例与方法

1. 病例:回顾性分析2007年8月至2014年9月我院贫血诊疗中心住院收治的TD-NSAA患者临床资料。参考德国AA研究组和欧洲SAA血液与骨髓移植工作组(EBMT SAA工作组)关于TD-NSAA的诊断标准[2],本研究TD-NSAA诊断标准为:符合Camitta NSAA标准[3],且外周血PLT<20×10^9/L或HGB<80 g/L或ANC<0.5×10^9/L。入组标准:前期未曾接受AA针对性治疗或应用CsA、雄激素不超过2周。排除标准:①先天性骨髓造血衰竭;②伴有明显临床或生化溶血检查证据的阵发性睡眠性血红蛋白尿(PNH)克隆;③伴有克隆性细胞遗传学异常;④因并发其他系统或脏器功能异常不能足量接受CsA治疗;⑤依从性不佳未能按方案完成至少3个月治疗;⑥加用了其他未知成分的单剂或混合制剂治疗。共纳入125例TD-NSAA患者,其中48例一线接受ATG/ALG联合CsA的标准IST方案,77例一线接受CsA联合雄激素治疗。

2. 治疗方法:猪源ALG(p-ALG,中国武汉生物制品所产品)20 mg·kg^{-1}·d^{-1}或兔源ATG(r-ATG,美国Genzyme公司产品)3.5 mg·kg^{-1}·d^{-1},静脉滴注,连用5 d。CsA起始剂量为3 mg·kg^{-1}·d^{-1},每12 h 1次分量口服。给药1周后检测CsA血药浓度并调整剂量使CsA血清谷浓度维持在150~250 μg/L,峰浓度维持在700~1 000 μg/L。雄激素为司坦唑醇片或达那唑片,司坦唑醇片用量儿童患者为2~4 mg/d,成人患者为6~8 mg/d,分次口服;达那唑片用量儿童患者为200~400 mg/d,成人患者为600 mg/d,分次口服。CsA及雄激素维持应用至患者获得血液学反应,血常规参数稳定至少3个月后开始缓慢减量,约每3个月减量1次,CsA每次减量25~50 mg/d,雄激素每次减量日总量的1/3,根据前次减量后患者病情决定减量间期和剂量。

3. 疗效判定:疗效判断标准参照文献[4]并增加良好部分反应(GPR),具体如下:①完全治疗反应(CR):HGB>100 g/L、PLT>100×10^9/L且ANC>1.5×10^9/L。②GPR:脱离血制品输注依赖,HGB>100 g/L、PLT>50×10^9/L且ANC>1.0×10^9/L。③部分治疗反应(PR):脱离血制品输注依赖,血常规指标未达GPR标准。④无治疗反应(NR):患者未脱离血制品输注依赖,血液学检查仍符合TD-NSAA标准,或疾病进展。CR、GPR、PR为获得血液学反应,CR、GPR为获得良好血液学反应。定义开始治疗后12个月内获得血液学反应者为有治疗反应。治疗开始至少3个月后未获治疗反应而接受二次IST或HSCT以及治疗12个月后获得治疗反应均视为一线治疗失败。复发定义为获得治疗反应且疗效维持时间至少达3个月患者血常规指标再次下降,需要恢复初始足量治疗,或再出现血制品输注依赖。患者因CsA减量过程中出现短暂血常规指标轻度下降,恢复CsA至前一剂量水平后血常规指标再恢复至原治疗反应水平,不视为复发。治疗3个月内死

亡定义为早期死亡，并视为未获治疗反应，纳入疗效评定分析。

4. 随访：在获得治疗反应前，每周至少检测2次血常规，每月进行肝肾功能检测，治疗后3、6及12个月进行骨髓细胞形态学、细胞遗传学检查及流式细胞术外周血细胞PNH克隆检测（2009年以前以粒细胞和成熟红细胞$CD55^+$、$CD59^+$细胞率小于95%为新发PNH克隆，2009年以后以Flaer方法出现>1%粒细胞或单核细胞阴性为新发PNH克隆）以评定疗效、监测疾病演变。此后至少每6个月随访1次。末次随访时间为2015年5月6日，中位随访33（0.4~93）个月。125例患者中20例失访，其中ATG/ALG联合CsA组失访6例，CsA联合雄激素组失访14例。总生存（OS）时间为诊断至患者死亡或末次随访时间；无事件生存（PFS）时间为诊断至发生任何事件或末次随访时间，事件包括：治疗无效、复发、新发克隆性染色体异常、溶血性PNH、MDS/AML转化、实体肿瘤、死亡。

5. 统计学处理：采用SPSS 22.0统计软件进行分析。基线特征中定量资料及脱离血制品输注时间的比较采用Mann-Whitney U检验，分类资料的比较采用行×列卡方检验；不同治疗组间早期死亡及治疗反应比较采用Fisher确切概率法；多因素分析采用Logistic回归；生存分析采用Kaplan-Meier法，以Log-rank检验比较组间差异。$P<0.05$为差异有统计学意义。

结　果

1. 基线特征：125例TD-NSAA患者中，男70例，女55例，男女比为1.27∶1；中位年龄27（6~66）岁。中位诊断至治疗间期为50（1~176）d。HGB中位数为67（27~117）g/L，ANC中位数为0.85（0.24~4.25）×10^9/L，PLT中位数为15（2~61）×10^9/L，网织红细胞绝对计数中位数为33.2（14.6~90.0）×10^9/L。单纯红细胞输注依赖24例（19.2%），单纯血小板输注依赖13例（10.4%），红细胞与血小板均输注依赖84例（67.2%），ANC<0.5×10^9/L同时红细胞输注依赖4例（3.2%）。48例接受ATG/ALG联合CsA方案治疗，77例接受CsA联合雄激素治疗，两组患者基线特征比较见表1。

2. 治疗反应比较：接受ATG/ALG联合CsA治疗的48例TD-NSAA患者中，1例（2.1%）治疗后第12天因肺出血死亡。两种不同治疗方案治疗后3个月和6个月患者治疗反应情况见表2，ATG/ALG联合CsA组血液学反应与良好血液学反应率均明显高于CsA联合雄激素组。治疗后6个月ATG/ALG联合CsA组获得血液学反应的36例患者脱离血制品输注依赖的中位时间为36.5（0~149）d，明显短于CsA联合雄激素组的98（14~180）d（$P<0.001$）；其中脱离红细胞输注依赖中位时间分别为26（0~123）d和90（6~180）d（$P<0.001$），脱离血小板输注依赖的中位时间分别为32（0~149）d和83（14~180）d（$P<0.001$）。

进一步比较不同输血依赖类型患者接受两种方案治疗后的治疗反应情况，结果见表3，单纯红细胞输注依赖患者和单纯血小板输注依赖患者接受ATG/ALG联合CsA与CsA联合雄激素治疗的血液学反应率及良好血液学反应率差异均无统计学意义；而在红细胞与血小板均输注依赖的患者中，ATG/ALG联合CsA组血液学反应率和良好血液学

表1　不同治疗方案组输血依赖非重型再生障碍性贫血患者基线特征比较

基线特征	ATG/ALG+CsA组（48例）	CsA+雄激素组（77例）	P值
中位年龄［岁，M（范围）］	21（6~66）	32（16~66）	<0.001
性别（男/女）	24/24	46/31	0.286
中位诊断至治疗间期［d，M（范围）］	55（14~145）	46（1~176）	0.292
单纯红细胞输注依赖［例（%）］	7（14.6）	17（22.1）	0.301
单纯血小板输注依赖［例（%）］	8（16.7）	5（6.5）	0.080
红细胞和血小板均输注依赖［例（%）］	33（68.8）	51（66.2）	0.771
中位HGB［g/L，M（范围）］	66.5（41~99）	69（27~117）	0.861
中位ANC［×10^9/L，M（范围）］	0.64（0.51~1.80）	1.04（0.24~4.25）	<0.001
中位PLT［×10^9/L，M（范围）］	13（2~30）	16（5~61）	<0.001
中位Ret［×10^9/L，M（范围）］	32.5（20.0~90.0）	33.3（14.6~59.5）	0.448

注：Ret：网织红细胞绝对值；ATG/ALG：抗人胸腺/淋巴细胞球蛋白；CsA：环孢素A

表2 两种不同治疗方案组输血依赖非重型再生障碍性贫血患者早期死亡及治疗反应比较[例数(%)]

组别	例数	早期死亡	3个月治疗反应		6个月治疗反应	
			血液学反应	良好血液学反应	血液学反应	良好血液学反应
ATG/ALG+CsA组	48	1(2.1)	34(70.8)	13(27.1)	36(75.0)	20(41.7)
CsA+雄激素组	77	0	35(45.4)	8(10.4)	43(55.8)	17(22.1)
*P*值		0.384	0.006	0.015	0.031	0.020

注:ATG/ALG:抗人胸腺/淋巴细胞球蛋白;CsA:环孢素A

表3 不同输血依赖类型非重型再生障碍性贫血患者接受不同方案治疗后6个月治疗反应的比较[例数(%)]

组别	例数	血液学反应	*P*值	良好血液学反应	*P*值
单纯红细胞输注依赖			1.000		0.356
ATG/ALG+CsA组	7	5(71.4)		4(57.1)	
CsA+雄激素组	17	12(70.6)		5(29.4)	
单纯血小板输注依赖			0.510		1.000
ATG/ALG+CsA组	8	7(87.5)		4(50.0)	
CsA+雄激素组	5	3(60.0)		3(60.0)	
红细胞和血小板均输注依赖			0.031		0.031
ATG/ALG+CsA组	33	24(72.7)		12(36.4)	
CsA+雄激素组	51	25(49.0)		7(13.7)	

注:ATG/ALG:抗人胸腺/淋巴细胞球蛋白;CsA:环孢素A

反应率均高于CsA联合雄激素组(*P*值均为0.031)。

3. 治疗后6个月影响血液学反应相关因素分析:按照性别、不同治疗方案分组;年龄,诊断至治疗间期,基线HGB、ANC、PLT、Ret按照中位数进行分组。对TD-NSAA患者各因素与治疗后6个月血液学反应进行单因素分析,结果见表4,以*P*≤0.1为入方程条件,PLT与治疗方案显示对6个月血液学反应有影响。将上述因素纳入多参数Logistic回归,结果显示ATG/ALG联合CsA[*OR*=3.931(95%*CI* 1.594~9.691),*P*=0.003]、基线PLT≥15×10^9/L[*OR*=4.282(95%*CI* 1.819~10.077),*P*=0.001]为获得6个月血液学反应的独立影响因素。

4. 疾病复发及克隆演变:获得血液学反应的86例患者中3例复发,均为CsA不能耐受或自行过快减量所致,复发率为3.5%,其中ATG/ALG联合CsA组2例(2/42,4.8%),CsA联合雄激素组1例(1/44,2.3%),差异无统计学意义(*P*=0.612)。

48例ATG/ALG联合CsA组患者中,1例PR患者治疗后15个月进展为骨髓增生异常综合征难治性血细胞减少伴有多系发育异常(MDS-RCMD);1例于治疗后3个月出现47, XY, +?Y[3]/46, XY[13]克隆性染色体异常,治疗后6个月染色体核型恢复正常,考虑为一过性细胞遗传学异常;6例新发PNH克隆,无溶血性贫血特征性临床表现或生化溶血检查证据。77例CsA联合雄激素患者中,无一例出现MDS转化和细胞遗传学异常克隆演变;5例新发PNH克隆,其中4例无临床或生化溶血证据,1例为经典型PNH。比较两治疗方案组PNH克隆演变发生率,差异无统计学意义(12.5%对6.5%,*P*=0.332)。

5. 生存分析:ATG/ALG联合CsA组和CsA联合雄激素组患者中位随访时间分别为35(8~93)个月、31(5~65)个月。ATG/ALG联合CsA组患者预期3年OS及PFS率分别为97.9%、71.2%,CsA联合雄激素组分别为100.0%、59.5%,差异均无统计学意义(OS:χ^2=1.458,*P*=0.227;PFS:χ^2=1.462,*P*=0.227)。

讨 论

NSAA临床表现异质性极大,部分患者仅轻微血细胞减少,血液学参数长时间保持稳定,生活质量良好;部分患者则外周血细胞缓慢进行性下降,逐渐出现输血依赖或进展到SAA[5]。对于非输血依赖NSAA患者采用"等与看"、不须特殊治疗干预的策略还未达共识,但对于TD-NSAA患者需积极治疗则少有争议[5-6]。前瞻性随机临床试验已经证明,IST可改善TD-NSAA患者血细胞减少、缓解输血依赖。与单用CsA比较,ATG联合CsA的IST可获得

表4 125例输血依赖非重型再生障碍性贫血患者治疗后6个月血液学反应的单因素分析[例数(%)]

因素	例数	血液学反应	χ^2值	*P*值
年龄			1.902	0.168
<27岁	59	41(69.5)		
≥27岁	66	38(57.6)		
性别			0.215	0.643
男	70	43(61.4)		
女	55	36(65.4)		
诊断至治疗间期			0.332	0.565
<50 d	61	37(60.6)		
≥50 d	64	42(65.6)		
HGB			0.723	0.395
<67 g/L	59	35(59.3)		
≥67 g/L	66	44(66.7)		
ANC			0.289	0.591
$<0.85\times10^9$/L	61	40(65.6)		
$\geq0.85\times10^9$/L	64	39(60.9)		
PLT			7.101	0.008
$<15\times10^9$/L	62	32(51.6)		
$\geq15\times10^9$/L	63	47(74.6)		
Ret			0.060	0.807
$<32.2\times10^9$/L	58	36(62.1)		
$\geq32.2\times10^9$/L	67	43(64.2)		
治疗方案			4.665	0.031
ATG/ALG+CsA	48	36(75.0)		
CsA+雄激素	77	43(55.8)		

注:ATG/ALG:抗人胸腺/淋巴细胞球蛋白;CsA:环孢素A

更高的血液学反应率(74%对46%,*P*=0.02)和更好的血液学反应质量[1]。基于此,英国血液学标准委员会推荐TD-NSAA一线治疗应采用ATG联合CsA方案[6]。

CsA单药治疗AA的疗效研究非常少。Gluckman等[7]对初治SAA患者单用ATG或CsA治疗,3个月无效进行交叉换药,3个月和12个月两组血液学反应率和OS率相当。Maschan等[8]报告13例儿童NSAA患者接受CsA单药治疗8周,其中11例(85%)获得血液学反应,4年实际OS率为85%。

ATG治疗须住院进行,常伴明显即刻输注不良反应和血清病反应,需更加积极的支持治疗,费用昂贵。临床实践中,除部分TD-NSAA患者采用英国血液学标准委员会指南[6]推荐的ATG联合CsA方案外,更多的患者接受CsA联合雄激素方案或雄激素、CsA单药治疗。迄今尚无进行前瞻性对照研究比较CsA联合雄激素治疗TD-NSAA是否可获得与指南推荐的ATG联合CsA方案相同的疗效。

雄激素可增强AA患者造血细胞端粒酶活性,提高造血细胞增殖能力,促进外周血细胞恢复[9],在残存造血细胞相对较多的NSAA中有可能改善外周血细胞计数[10]。文献报道使用雄激素治疗AA的疗效结果差异较大,Champlin等[11]报告SAA/VSAA患者接受ATG联合或不联合雄激素治疗并不明显增加血液学反应率;Bacigalupo等[12]的结果则表明加用雄激素尽管并不显著改善近期生存率,但可提高AA患者ATG治疗的血液学反应率(56%对40%,*P*=0.04),在女性和ANC≤0.5×10^9/L的患者中表现更为突出,而在纳入的与TD-NSAA相当的ANC>0.5×10^9/L患者中,ATG治疗基础上加用雄激素并未带来血液学反应率的提高(64%对61%,*P*=0.10)。Young等[13]报道的一项多中心临床研究显示,纳入的44例NSAA和慢性(病史>3个月)SAA患者中25例接受ATG治疗后3个月,7例(28%)获得血液学反应,而19例采用大剂量雄激素治疗者无一例获得血液学反应;Jiang等[14]采用ATG联合CsA及雄激素治疗儿童NSAA患者血液学反应率为83.3%,明显高于CsA联合雄激素治疗组的41.7%(*P*=0.006),前者CR率为27.8%,也明显高于后者的8.3%,ATG联合CsA及雄激素治疗组进展为SAA患者也明显低于后者(*P*=0.021)。

为减少NSAA异质性对结果的影响,我们仅回顾性分析TD-NSAA患者,结果显示ATG/ALG联合CsA治疗患者OS率与CsA联合雄激素组相当,但治疗后3个月、6个月血液学反应率和良好血液学反应率均明显高于CsA联合雄激素组,且ATG/ALG联合CsA组脱离血制品输注时间短于CsA联合雄激素组,生存质量更优。单因素和多因素分析也均显示治疗方案对6个月血液学反应率有显著影响,采用ATG/ALG联合CsA治疗获得血液学反应的概率是CsA联合雄激素组的3.93倍。

分层分析不同输血依赖类型中两种治疗方案对患者疗效的影响,我们发现在红细胞和血小板共同输注依赖的患者中,ATG/ALG联合CsA较CsA联合雄激素治疗可获得更高血液学反应率和更好血液学反应质量;而单一系列输血依赖患者两种治疗方法血液学反应率相近。雄激素可促进肾脏分泌EPO,刺激红系祖细胞增殖以增加红细胞生成[15-16]。考虑到单纯红细胞输注依赖和单纯血小板输注依赖患者样本数均较小,检出两种方案治疗反

应差异的效能过低，对本研究CsA联合雄激素与ATG/ALG联合CsA治疗单一系列TD-NSAA血液学反应差异无统计学意义的结论应慎重解释。

NSAA治疗的目的主要是延长生存期和改善生活质量。本研究结果虽然显示ATG/ALG联合CsA一线治疗TD-NSAA血液学反应率和良好血液学反应率均优于CsA联合雄激素组，但两种治疗方案患者3年OS率和PFS率并差异无统计学意义。我们认为这主要与近年支持治疗改善和本研究随访时间相对较短有关，未达良好血液学反应、甚至无治疗反应患者存活时间较以往明显延长。随着时间的延长，获得血液学反应者最终将会被证明有更高的生存机会。

综上，我们的回顾性研究结果表明，一线采用CsA联合雄激素方案治疗TD-NSAA，患者血液学反应率和血液学反应质量均不及ATG/ALG联合CsA方案治疗者；ATG的应用并未增加早期死亡率。提示TD-NSAA患者CsA联合雄激素治疗并非理想选择，应优选ATG/ALG联合CsA方案治疗。

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·论著·

ATG/ALG联合环孢素A治疗老年重型再生障碍性贫血16例疗效及安全性评估

李建平 杨文睿 李园 叶蕾 周康 井丽萍 李洋 彭广新 宋琳 张凤奎 张莉

【摘要】 **目的** 评估老年(≥60岁)重型再生障碍性贫血(SAA)患者接受兔抗人胸腺细胞球蛋白(rATG)/猪抗人淋巴细胞球蛋白(pALG)联合环孢素A(CsA)的强烈免疫抑制治疗(IST)方案的疗效和安全性。**方法** 回顾性分析16例老年SAA患者接受rATG/pALG+CsA治疗的血液学反应率和安全性,分析影响疗效的相关因素。**结果** 16例患者男13例,女3例,中位年龄63.5(60~79)岁,其中60~69岁13例,≥70岁3例;SAA患者9例,极重型AA(VSAA)患者7例;9例患者接受rATG治疗,7例患者接受pALG治疗。16例患者均顺利完成rATG/pALG治疗,治疗后早期死亡2例(12.5%),均为VSAA患者(2/7,28.6%);IST后6个月9例(56.3%)患者获得血液学反应,5例无治疗反应。9例应用rATG的患者有2例获得血液学反应,7例应用pALG患者全部获得血液学反应,差异有统计学意义(22.2%对100.0%,*P*=0.003)。rATG/pALG+CsA相关不良反应轻微,经对症治疗好转。**结论** 老年SAA接受rATG/pALG联合CsA的IST方案仍可获较好血液学反应;VSAA患者早期死亡率高,治疗风险大;pALG治疗老年SAA疗效可能优于rATG。

【关键词】 贫血,再生障碍性; 老年人; 免疫抑制法

Antithymocyte/Antilymphocyte globulin plus cyclosporine A therapy for the treatment of older patients with severe aplastic anemia

Li Jianping, Yang Wenrui, Li Yuan, Ye Lei, Zhou Kang, Jing Liping, Li Yang, Peng Guangxin, Song Lin, Zhang Fengkui, Zhang Li. Institute of Hematology and Blood Diseases Hospital, CAMS & PUMC, Tianjin 300020, China

Corresponding author: Zhang Li, Email: choli@medmail.com.cn

【**Abstract**】 **Objective** To evaluate the efficacy and safety of intensive immunosupressive therapy (IST) with antithymocyte/antilymphocyte globulin plus cyclosporine A in the treatment of older patients (≥60 years) with severe aplastic anemia (SAA). **Methods** The hematologic response and safety of sixteen older SAA patients treated with IST regimen in our hospital were retrospectively analyzed , and the factors affecting response were also explored. **Results** A total of 16 older SAA patients were involved, the median age was 63.5 (60-79) years. Among them, 7 were VSAA and 9 were SAA; 9 patients received Rabbit anti-human thymocyte globulin (rATG), and 7 patients porcine anti-human lymphocyte globulin (pALG). Two patients died within 3 months after IST; at the 6 months after IST, 9 patients achieved hematology response and 5 patients had no response; overall response rate was 56.3%. Two (22%) of the 9 patients treated with rATG achieved hematology response; However, all 7 patients (100.0%) treated with pALG achieved hematology response. rATG/pALG associated adverse reactions were mild and easily managed. **Conclusion** The older patients with SAA could still benefit from IST consisting of standard dose rATG/pALG with CsA, and the patients with VSAA had worse prognosis, pALG was inferior to rATG as a first treatment for SAA.

【**Key words**】 Anemia, aplastic; Older; Immunosupressive therapy

重型再生障碍性贫血(Severe aplastic anemia, SAA)临床表现为重度全血细胞减少和骨髓造血衰竭,若不能给予积极有效的治疗,大多数患者会在诊断后的1~2年内死于出血或感染并发症。抗胸腺/淋巴细胞球蛋白(ATG/ALG)联合环孢素A(CsA)是目前公认的不适合行造血干细胞移植(HSCT)的SAA患者的一线强烈免疫抑制治疗(IST)方案。ATG/ALG+CsA治疗可以使60%~80%的SAA患者恢复三系造血。与HSCT不同,年龄本

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作者单位:300020 天津,中国医学科学院、北京协和医学院血液学研究所、血液病医院

通信作者:张莉,Email:choli@medmail.com.cn

身不是IST的禁忌因素，其实施不受年龄限制[1]，国外报道行IST SAA患者最大年龄为89岁[2]。然而国内对老年SAA患者行IST相对保守，缺少应用经验。为了解老年SAA患者行IST的有效性和安全性，我们回顾性总结了我院2006年10月至2014年10月16例接受IST的老年SAA患者的临床特征、IST的耐受性和疗效，初步分析了影响疗效的预后因素，以期对老年SAA患者的治疗提供临床依据。

病例与方法

1. 病例：初治老年（年龄≥60岁）SAA/极重型AA（VSAA）患者16例，均为2006年10月至2014年10月我中心住院治疗患者。AA诊断依据1987年国际粒细胞减少和AA诊断标准[3]，严重程度分型参照Camitta标准[4]。所有患者均行染色体检查、外周血细胞流式细胞术CD55、CD59或嗜水气单胞菌溶素变异体（Flaer）检测排除低增生性骨髓增生异常综合征（MDS）及阵发性睡眠性血红蛋白尿症（PNH）。所有患者均无重要脏器功能严重异常。

2. IST方案：兔抗人ATG（rATG，美国健赞公司产品）1.75~3.50 mg·kg^{-1}·d^{-1}或猪抗人ALG（pALG，武汉生物制品研究所产品）20 mg·kg^{-1}·d^{-1}静脉输注，连用5 d；同时给予泼尼松1 mg·kg^{-1}·d^{-1}预防即刻不良反应和血清病反应，2周后泼尼松开始减量，3周停药。出现血清病反应者给予氢化可的松100 mg/d静脉滴注。患者疾病诊断明确后即开始CsA口服，以3 mg·kg^{-1}·d^{-1}分两次口服起始，1周后检测血药浓度，调整CsA剂量维持全血谷浓度200~400 μg/L。口服CsA至少6个月，当达到最佳血液学反应并血液学参数稳定至少3个月后开始缓慢减量，每3个月约减量1 mg·kg^{-1}·d^{-1}。口服CsA治疗共2~3年。

3. 支持治疗：ANC＜0.5×10^9/L时采用G-CSF 300 μg/d皮下注射，HGB<70 g/L及PLT<10×10^9/L时分别给予红细胞、血小板输注支持治疗。

4. 随访及疗效判定：随访截止日期为2015年4月30日。所有患者分别于IST后3、6、9、12个月进行随访，此后每年随访1次，观察包括外周血和骨髓细胞形态学及组织活检、PNH克隆、脏器功能、免疫指标等以评估血液学反应和不良反应。治疗后3个月内死亡定义为早期死亡，纳入治疗相关不良反应及疗效评价分析。疗效判定采用Camitta标准[4]。脱离红细胞和血小板输注，HGB≥100 g/L、ANC≥1.5×10^9/L且PLT≥100×10^9/L判定为完全治疗反应（CR）；脱离红细胞和血小板输注，ANC>0.5×10^9/L，实验室检查不再符合SAA标准而未达CR标准者判定为部分治疗反应（PR）；未达PR标准者为无治疗反应（NR）。生存时间定义为开始IST至患者死亡或随访终点。

5. 统计学处理：使用SPSS 19.0软件进行统计学分析，计量资料组间比较采用非参数检验，不同组间6个月血液学反应率的单因素分析采用卡方检验。P<0.05为差异有统计学意义。

结　果

1. 患者基本特征：16例初诊的老年SAA/VSAA患者，男13例，女3例，中位年龄63.5（60~79）岁；其中60~69岁13例，≥70岁3例。中位ANC 0.41（0.02~2.60）×10^9/L，中位网织红细胞绝对值（ARC）14.9（2.8~32.0）×10^9/L，中位PLT 15（6~18）×10^9/L；AA发病至开始IST中位时间为43（29~390）d；VSAA患者7例，SAA患者9例。所有患者IST前均进行PNH筛查，其中3例患者存在微小PNH克隆，无临床溶血表现和生化检查溶血证据；2例患者染色体核型为45,X,-Y，其余患者为正常核型；1例患者AA发病前2年诊断胃癌并行胃大部切除术。9例患者接受rATG治疗，7例患者接受pALG治疗。

2. 早期死亡：16例患者中2例（12.5%）在治疗后3个月内死亡，均为VSAA患者（2/7，28.6%），死亡原因亦均为重症感染。

3. 血液学反应：IST后6个月9例患者获得血液学反应，其中5例CR，4例PR；5例NR，总体血液学反应率56.3%。获得血液学反应的9例患者，脱离红细胞输注中位时间33（0~85）d，脱离血小板输注中位时间36（0~94）d。

4. 影响疗效的因素：单因素分析结果显示性别、年龄、发病至开始IST的时间、初始PLT及ARC等对IST后6个月血液学反应无明显影响。分析骨髓造血衰竭严重程度对疗效的影响，VSAA患者7例，早期死亡2例（28.6%），3例获得血液学反应，2例NR；SAA患者9例，无早期死亡，6例获得血液学反应，3例NR，血液学反应率分别为42.8%和66.7%（P=0.615）。分析免疫抑制剂类型对疗效的影响，9例应用rATG患者中，2例早期死亡（22.2%），2例PR（分别于治疗后12个月和24个月达CR），5例NR，总体血液学反应率为22.2%；7例应用pALG患者中，无一例早期死亡，5例CR，2例PR，总血液学反应率为100.0%（7/7），明显高于应用rATG患者（P=

0.003)。13例60~69岁患者,1例早期死亡,9例获得血液学反应,3例NR;3例≥70岁患者,1例早期死亡,2例NR(*P*=0.063)。

5. IST后淋巴细胞恢复:rATG/pALG两组患者IST前后淋巴细胞绝对值(ALC)变化见图1,结果显示rATG与pALG治疗后患者外周血ALC均明显降低,而以rATG治疗后下降更为明显,ALC低下维持时间更长、恢复更慢,至治疗后12周仍未能恢复到治疗前水平。

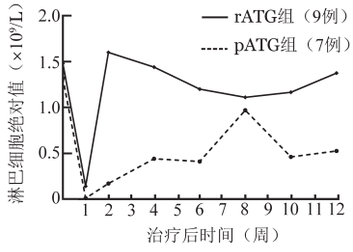

图1 16例老年再生障碍性贫血患者不同免疫抑制剂类型治疗后淋巴细胞绝对值变化

6. 不良反应:IST期间药物相关不良反应包括:药物性发热3例,糖皮质激素致血压升高2例、血糖升高3例,停药后症状消失;血清病反应7例,经对症治疗好转。9例患者合并感染,早期死亡2例,1例于IST后12个月死于反复感染,其余患者经治疗后好转。未出现严重出血和肝肾功能损害,无心脑血管意外发生。无严重不良反应发生。

排除早期死亡2例,剩余14例患者中,2例不能耐受,CsA剂量未达治疗标准(CsA血药浓度持续低于200 μg/L);12例CsA血药浓度浓度达到200~250 μg/L,其中6例在治疗后6~24个月出现肾功能异常(其中3例发生于CsA血药浓度300~450 μg/L时),血肌酐最高者为152 μmol/L,CsA减量后肌酐恢复正常水平,减量后CsA浓度在150 μg/L左右,其中5例获得血液学反应,另6例治疗全程中无肾功能异常。

7. 异常克隆转归:合并微小PNH克隆患者3例,治疗后NR、PR、CR各1例,微小PNH克隆均持续存在,无一例发生溶血。－Y患者2例,1例NR,异常克隆持续存在;1例CR,异常克隆于治疗后6个月消失。

8. 生存及随访结果:16例患者中位随访18(3~75)个月,2例早期死亡;1例IST后12个月死于反复感染;1例在IST后6个月失访;3例治疗无效患者持续输血依赖;另9例患者生存状况良好无复发,12个月累积生存率为77.8%。2例患者在IST后3个月出现新发克隆性染色体异常,分别为－Y和t(1;13),随访期内2例患者骨髓象无血细胞发育异常形态改变,血常规检查提示持续CR;1例染色体正常核型NR患者IST后20个月转化为典型MDS-难治性贫血伴原始细胞过多,持续输血依赖。2例患者IST后6个月出现新发PNH克隆,无溶血表现。1例既往胃癌患者无肿瘤复发迹象,病情稳定。

讨　论

AA可发生于任何年龄段,而10~20岁和≥60岁为两个发病高峰[5]。随着对疾病认识和临床研究的进展,HSCT和IST已经成为SAA患者标准的可选择方案。鉴于年龄>50岁的患者移植相关死亡率大大增高[6],而行IST仍可有60%左右的生存率[7],因此欧洲血液学标准委员会推荐年龄>50岁的患者首选ATG联合CsA的强烈IST。在国内,老年SAA患者治疗方案选择更为保守,行ATG/ALG联合CsA者甚少,资料缺乏。因此我们总结了我中心行IST的老年患者的疗效及安全性,以提高对老年SAA治疗和预后的认识。

本组16例患者年龄大,造血衰竭严重,其中7例患者符合VSAA标准,但身体状况尚好,无重大基础疾病。治疗后6个月的总体血液学反应率为56.3%,其中大部分为良好血液学反应,生存质量得到改善,其血液学反应率与国际报道相当。Tichelli等[2]研究显示≥60岁AA患者IST疗效约为52%,与20~49岁和50~59岁年龄组比较差异无统计学意义(*P*=0.055),提示SAA患者IST疗效并不依赖于年龄。考虑到标准治疗剂量ATG本身的不良反应和可能增加老年患者的病死率,也有研究者应用低强度免疫抑制剂以提高长期生存率,Killick等[8]单独应用低剂量(1/3标准剂量)马ATG,纳入了14例老年SAA患者,无一例发生治疗相关死亡,12例可评价疗效患者中仅1例有效,提示低剂量ATG耐受性较好,但疗效不佳,并不支持老年AA患者应用低剂量ATG,推荐应用标准剂量IST。

老年患者行IST较年轻患者出现严重感染和出血的风险大,Tichelli等[2]的研究显示,接受IST 3个年龄组AA患者中≥60岁组5年生存率最低,为50%(*P*<0.01);病死率最高,为32%(*P*=0.029)。2008年Kao等[9]的研究显示24例老年AA患者中,6例应用标准剂量的ATG + CsA治疗,13例应用减低剂量ATG + CsA治疗。结果显示标准剂量组早期死亡率

为43%，减低剂量组为18%（*P*=0.4）；而标准剂量组血液学反应率为43%，减低剂量组为53%（*P*=0.4）。影响死亡的原因包括疾病严重程度、感染及伴随疾病（冠心病、心力衰竭、肝硬化、脑血管疾病等）。提示此类患者适度减低IST强度仍可获得疗效，或可能减少早期死亡，而治疗前对患者疾病及身体状况评估非常重要。本组16例患者除了2例ATG减量，大部分均应用常规剂量rATG/pALG，输注过程均顺利，无严重不良反应发生。IST后有2例早期死亡，12个月累积生存率为77.8%，表明老年患者对药物的耐受较好，治疗相关死亡率并不高。这可能与本组患者基础身体状况较好有关。

老年患者长期应用CsA引起的肾功能异常需要重视。本组患者中57%（8/14）出现肾功能异常，调整CsA剂量后肾功能均获得好转，尽管减量后CsA血药谷浓度达不到200~400 μg/L，仅在150 μg/L的水平，也未明显影响疗效。因此，针对老年人的最佳CsA血药谷浓度还需要进一步摸索。

本组VSAA与SAA患者疗效差异无统计学意义，但早期死亡患者均为VSAA患者（2/7，28.6%）。另一个可能影响疗效的因素为免疫抑制剂类型，从血液学反应率看pALG组明显优于rATG组（*P*=0.003）。Scheinberg等[10]的随机对照研究显示马与兔ATG 6个月血液学反应率分别为68%对37%（*P*<0.01），但相关研究显示，rATG较马ATG有更强的免疫抑制作用，提示更强的IST并不意味着更好的疗效[11-13]。但是，这些研究纳入患者中位年龄多在20~40岁，≥60岁患者相关研究较少。在我们的研究中rATG组IST血液学反应率较低（22.2%，2/9）；而pALG组血液学反应率达100.0%（7/7）。同时，两组患者强烈IST前后ALC变化的统计分析显示，rATG组较pALG组ALC下降更多，恢复更慢；rATG较pALG有更强的免疫抑制作用，可能导致更多的感染、早期死亡，从而导致IST总体疗效不佳。但由于本组患者病例数较少，且为单中心回顾性分析，这些初步发现尚需前瞻性、多中心研究来加以确认。

综上，老年SAA患者进行ATG/ALG+CsA方案的IST耐受性较好，疗效也与60岁以下患者相当；但老年VSAA患者由于造血衰竭重于SAA患者，早期死亡率高，治疗风险较大。

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ORIGINAL ARTICLE

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Efficacy and safety of porcine ALG compared to rabbit ATG as first-line treatment for children with acquired aplastic anemia

Yangmin Zhu | Yang Yang | Wenrui Yang | Lin Song | Yuan Li | Huihui Fan | Yang Li | Jianping Li | Lei Ye | Xin Zhao | Kang Zhou | Guangxin Peng | Liping Jing | Li Zhang | Fengkui Zhang

Department of Therapeutic Center of Anemia, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College (CAMS & PUMC), Tianjin, China

Correspondence
Fengkui Zhang, Department of Therapeutic Center of Anemia, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College (CAMS & PUMC), Tianjin, China.
Email: zhangfengkuixys@163.com

Abstract

Objective: To assess the outcomes of children with acquired aplastic anemia (AA) treated in China with first-line porcine anti-lymphocyte immunoglobulin (p-ALG)/rabbit anti-thymocyte immunoglobulin (r-ATG) combined with cyclosporine A (CSA).

Methods: We performed a single-center, non-randomized, retrospective cohort study to assess the outcomes of 189 children with AA treated in China with first-line p-ALG/r-ATG combined with CSA between 2014 and 2018.

Results: No significant differences were observed in the overall response rates at 3, 6, 12, or 24 months (3 months: 61.9% vs 67.4%, $P = .5$; 6 months: 70.9% vs 73.9%, $P = .69$; 12 months: 77.3% vs 73.3%, $P = .58$; 24 months: 81.6% vs 78.6%, $P = .59$) after either p-ALG- or r-ATG-based immunosuppressive therapy. No significant differences were observed in overall survival or failure-free survival between the p-ALG group and the r-ATG group.

Conclusion: Our results reveal that the therapeutic efficacy and safety of p-ALG combined with CSA did not differ significantly from those of r-ATG combined with CSA as first-line therapy for pediatric patients with AA. Moreover, p-ALG has the advantage of significantly lower cost compared with r-ATG.

KEYWORDS

anti-lymphocyte immunoglobulin, anti-thymocyte immunoglobulin, aplastic anemia, efficacy and safety, immunosuppressive therapy

1 | INTRODUCTION

Acquired aplastic anemia (AA) is a rare and life-threatening hematological disease characterized by pancytopenia and hypocellular bone marrow. Severe aplastic anemia (SAA) and very severe aplastic anemia (VSAA) are associated with high morbidity and mortality. The incidence of AA in Europe and North America is approximately two per million per year, while the incidence is two to three times higher in Asia.[1] AA is regarded as the result of autoimmune-mediated destruction of hematopoietic cells, as demonstrated by successful treatment with immunosuppressive therapy (IST), leading to the relief of symptoms or complete recovery of peripheral blood counts in a percentage of patients.[2] Matched sibling hematopoietic stem cell transplantation (MSD-HSCT) is considered the first choice of therapy for patients with newly diagnosed SAA or VSAA who are <35-50 years of age. First-line IST is indicated for patients who are >35-50 years of age or those who lack a matched sibling donor.[3]

The current standard first-line IST is horse ATG (h-ATG) combined with cyclosporine A (CSA). A few prospective randomized

trials have compared horse ATG (h-ATG) with rabbit ATG (r-ATG) and found it to be superior in terms of short-term response and long-term survival.[4,5] Furthermore, some studies have demonstrated that r-ATG is inferior to h-ATG for treating AA in children.[6,7] Other studies, although not prospective, have failed to show significant differences between h-ATG and r-ATG.[8,9] However, h-ATG is not available in some countries, including China, due to manufacturing difficulties. Therefore, r-ATG has been used as the first-line IST regimen in patients with AA.

Porcine anti-lymphocyte immunoglobulin (p-ALG) was approved by the State Food and Drug Administration of China as a therapeutic agent in 2004. It is 40% cheaper than r-ATG and has been widely used as an IST agent for AA in China.[10,11] Despite its extensive use, no studies have compared the efficacy and safety of p-ALG vs r-ATG combined with CSA as a first-line IST for children with AA. In this retrospective study, we analyzed the response rates, adverse events and toxicity, and long-term survival of 189 pediatric patients with AA treated with p-ALG or r-ATG plus CSA.

2 | METHODS

2.1 | Patients

This was a single-center, non-randomized, retrospective cohort study that assessed the outcomes of a large number of children with AA treated in China with first-line p-ALG/r-ATG combined with CSA between January 2014 and December 2018. This study was approved by the Ethical Committee of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College. Appropriate informed consent was obtained from all patients and/or their legal guardians in accordance with the Declaration of Helsinki. AA was defined by at least two of the following: hemoglobin concentration <100 g/L, platelet count < 50×10^9/L, neutrophil count < 1.5×10^9/L, and hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis on bone marrow biopsy; VSAA, SAA, and non-severe AA (NSAA) were defined by standard criteria.[3]

The inclusion criteria were as follows: patient ≤16 years of age diagnosed with SAA or transfusion-dependent NSAA; no prior IST, except for CSA with or without androgens and corticosteroids and with or without intravenous immunoglobulin; and ineligible for an HLA-identical sibling donor HSCT or voluntarily chose IST, even if eligible for an HLA-matched sibling donor transplant. Exclusion criteria were as follows: diagnosis of Fanconi anemia, dyskeratosis congenita, or other congenital bone marrow failure syndrome; evidence of myelodysplastic disease; paroxysmal nocturnal hemoglobinuria (PNH) and evidence of significant hemolysis; severe uncontrolled infection or unexplained fever >38.5°C; and severe complications of the liver, renal, heart, or metabolic systems with a life expectancy <3 months.

2.2 | Treatment protocol

2.2.1 | Immunosuppressive regimens

The two treatment regimens (p-ALG or r-ATG combined with CSA) were offered to patients without related HLA-matched donors, or patients who voluntary chose IST even if eligible for an HLA-identical sibling donor HSCT. The final treatment decision was made based mainly on the patient's preference. More than 200 patients with AA received IST in our center each year. All patients were housed in sterile rooms with strict isolation from the start of therapy. Patients in the p-ALG group received 20 mg/kg/day p-ALG (Wuhan Institute of Biological Products Co., Ltd.) for five consecutive days, and patients in the r-ATG group received 3 mg/kg/day r-ATG (thymoglobulin, Genzyme) for five consecutive days. Prednisolone (1 mg/kg/day) was administered with the dose tapered over 1 month to prevent serum sickness. The CSA dose was 3-5 mg/kg per day administered from day 1 or diagnosis until the best response, then held for 3 months, followed by a slow taper until discontinuation. For partial response patients, if no further response was achieved for 3 months in full dose of CSA treatment, then the best response was defined. If CSA dependence developed, then CSA was held until the last follow-up. The blood trough concentration was monitored and maintained between 150 and 250 ng/ML during CSA treatment before tapering.

2.2.2 | Supportive therapies and further treatment

Red cell and platelet transfusions were administered to maintain safe blood counts according to institutional guidelines. Granulocyte-colony-stimulating factor (5 μg/kg/day) was administered to neutropenic patients to maintain neutrophils >0.5×10^9/L to avoid an infection. Other hematopoietic growth factors, such as recombinant human thrombopoietin (rhTPO, 300 U/kg/day), were administered on the decision of the physician due to uncertain effectiveness, related costs and platelet supply. Prophylactic antifungal and antiviral agents were administered according to our institutional guidelines. Non-responsive or relapsed patients were treated with a second course of IST, eltrombopag, or a blood transfusion as supportive care. Patients who underwent transplantation as a rescue therapy were also included in the analysis in both groups.

2.2.3 | Response evaluation

Each patient was re-examined every 3-6 months after IST. We assessed the patient's clinical manifestations and re-examined their complete blood cell count, hepatic and renal function, CSA concentration, PNH clone, or other clinical or laboratory evidence of clonal disease (including myelodysplastic syndrome and leukemia). Bone marrow smears and biopsies, as well as cytogenetic analyses, were re-examined according to clinical indications. Response was defined

according to established criteria and was evaluated until the patient received further treatment.[3] The primary endpoints were a response at 3, 6, 12, and 24 months after IST, and secondary endpoints were transplant-free survival (TFS, calculated from the date of IST to the day of either transplantation or death or last follow-up), Failure-free survival (FFS, defined as survival with response or last follow-up; IST treatment failure included no response within 12 months, relapse, disease progression requiring a second course of IST or salvage allo-HSCT or other intervention, clonal evolution, and death), and overall survival (OS, calculated from the date of IST to the day of death or the last follow-up) 5 years after IST. Early death was defined as a patient who died within 3 months of IST. CSA dependence was defined as a recurrence of any cytopenia after reducing or stopping CSA, followed by recovery when CSA was increased or resumed.

2.3 | Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (IBM SPSS). Comparisons between treatment groups were performed using the chi-square test for categorical variables and the independent samples *t* test for variables with a normal distribution and equal variances. The non-parametric Mann-Whitney *U* test was used for variables with a significant difference in distribution. A univariate survival analysis was conducted using the Kaplan-Meier method, and survival was compared using the log-rank test. A multivariate survival analysis was performed using the Cox proportional hazard model. Significant variables among those assessed in univariate analyses were considered for the multivariate model. A *P*-value <.05 was considered significant.

3 | RESULTS

3.1 | Patient characteristics

In total, 189 consecutive AA patients who were ≤16 years old received IST with p-ALG/r-ATG combined with CSA between January 2014 and November 2018. The patient characteristics are shown in Table 1. No significant differences in demographic or clinical features were observed between the two groups. The median follow-up for the p-ALG group was 1370 (range, 86-2025) days and that for the r-ATG group was 1416 (range, 22-2027) days.

TABLE 1 Patient characteristics

Characteristics, n (%)	p-ALG (n = 142)	r-ATG (n = 47)	*P*-value
Median age, y (range)	12 (4-16)	11 (5-16)	NS
Sex (male/female)	81/61	25/22	NS
Etiology			
Idiopathic	134 (94)	45 (96)	NS
Posthepatitis	8 (6)	2 (4)	NS
Disease severity			
VSAA	48 (34)	16 (34)	NS
SAA	80 (57)	29 (62)	NS
NSAA	14 (10)	2 (4)	NS
Blood cell counts			
Hemoglobin (g/L, mean ± SD)	74.9 ± 16.4	78.3 ± 18.3	NS
Neutrophil count ($\times10^9$/L, mean ± SD)	0.63 ± 0.7	0.70 ± 0.95	NS
Platelet count ($\times10^9$/L, mean ± SD)	24.0 ± 17.6	25.8 ± 18.8	NS
Reticulocyte count ($\times10^9$/L, mean ± SD)	25.6 ± 22.9	21.9 ± 17.7	NS
Abnormal cytogenetic clone	8 (6)	3 (6)	NS
PNH clone at diagnosis	13 (9)	8 (17)	NS
Median clone size			
Red cells	0 (0-45.1)	0 (0-1)	ND
Granulocytes	4.8 (1.3-65.3)	2.6 (1.2-27.6)	ND
Previous treatment			
CSA ± androgen	120 (85)	41 (87)	NS
Corticosteroids ± IVIg	18 (13)	7 (15)	NS
rhTPO treatment	90 (63)	33 (70)	NS
G-CSF treatment	138 (97)	47 (100)	NS
Median duration of CSA treatment, d (range)	13 (0-1,642)	12 (0-130)	NS
Median disease duration, d (range)	30 (3-2,737)	30 (5-1,825)	NS
Median time from diagnosis to IST, d (range)	14 (1-2,190)	14 (2-130)	NS
Median follow-up, d (range)	1370 (86-2,025)	1416 (22-2,027)	NS

Abbreviations: ND, not done; NS, not significant.

3.2 | Response

No significant differences were observed in the overall response (OR) rates at 3, 6, 12, or 24 months (3 months: 61.9% vs 67.4%, *P* = .5; 6 months: 70.9% vs 73.9%, *P* = .69; 12 months: 77.3% vs 73.3%, *P* = .58; 24 months: 81.6% vs 78.6%, *P* = .59) after either p-ALG- or r-ATG-based IST (Table 2 and Figure 1). No differences were observed in the complete response (CR) rates between the p-ALG and r-ATG groups (3 months: 8.5% vs 6.5%, *P* = .92; 6 months: 15.6% vs 17.4%, *P* = .77; 12 months: 34.3% vs 40%, *P* = .49; 24 months: 57% vs 54.7%, *P* = .8). We then analyzed the median intervals between IST and response and found no significant differences between the p-ALG (40, range 2-550 days) and r-ATG (38, range 7-100 days) groups (*P* = .73). The median times to achieve transfusion independence of red blood

TABLE 2 Response to porcine anti-lymphocyte immunoglobulin (p-ALG) vs rabbit anti-thymocyte immunoglobulin (r-ATG) as first-line therapy in children with acquired aplastic anemia

Time	3 mo		6 mo		12 mo		24 mo	
Response, n (%)	p-ALG	r-ATG	p-ALG	r-ATG	p-ALG	r-ATG	p-ALG	r-ATG
PR	76 (54)	28 (61)	78 (55)	26 (57)	59 (43)	15 (33)	29 (25)	10 (24)
CR	12 (8)	3 (7)	22 (17)	8 (17)	47 (34)	18 (40)	65 (57)	23 (55)
NR	54 (38)	15 (33)	41 (29)	12 (26)	31 (23)	12 (27)	20 (18)	9 (21)
OR	88 (62)	31 (67)	100 (71)	34 (74)	106 (77)	33 (73)	93 (82)	33 (79)
Relapse	0	0	0	0	1 (1)	1 (3)	2 (2)	0
Transplanted	0	0	1 (1)	1 (2)	2 (2)	4 (10)	3 (3)	2 (6)
Death	0	1 (2)	0	0	3 (2)	0	6 (6)	0
Total	142	46	141	46	137	45	114	42

Abbreviations: NR, no response; PR, partial response.

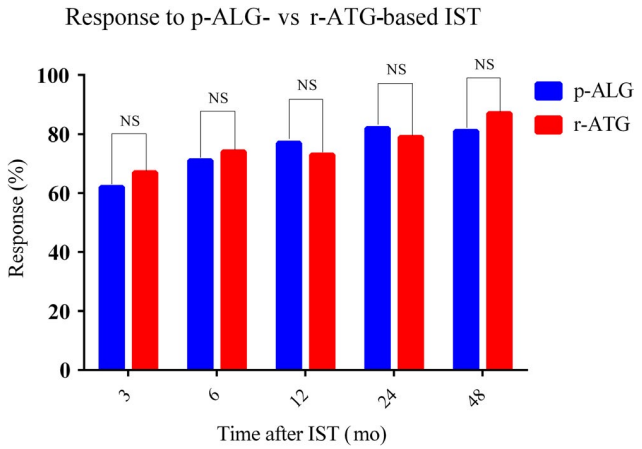

FIGURE 1 Response to porcine anti-lymphocyte immunoglobulin (p-ALG)- and rabbit anti-thymocyte immunoglobulin (r-ATG)-based immunosuppressive therapy at 3, 6, 12, 24, and 48 mo after treatment

cells were 33 (range 0-495) days for the p-ALG group and 34 (range 0-93) days for the r-ATG group (P = .71). The median times to achieve transfusion independence of platelets were 38 (range 0-550) days for the p-ALG group and 34 (range 1-100) days for the r-ATG group (P = .64).

Next, we explored potential factors influencing OR rates. The absolute reticulocyte count (ARC) at 1 month significantly affected OR rates at 12 months (70.2% for ARC ≤ 40 × 10^9/L at 1 month vs 82.8% for ARC > 40 × 10^9/L at 1 month, P = .047). OR rates at 12 months tended to differ in terms of the rhTPO treatment (80.3% for rhTPO treatment vs 68.3% for no rhTPO treatment, P = .07). No significant differences in OR rates were observed at 12 months between the two groups in term of the ARC at disease diagnosis (75% for ARC ≤ 15 × 10^9/L at disease diagnosis vs 77.4% for ARC > 15 × 10^9/L at disease diagnosis, P = .71), sex (78.4% for male vs 73.8% for female, P = .46), age (78.2% for age ≤ 12 years vs 74.1 for age > 12 years, P = .51), disease severity (71.7% for VSAA vs 78.5% for SAA, P = .32; 78.5% for SAA vs 80% for NSAA, P = .83), disease duration (75.8% for disease duration ≤30 days vs 77.1% for disease duration >30 days, P = .83), disease etiology (75.7% for idiopathic vs 88.9% for posthepatitis, P = .61), abnormal cytogenetic clone (70% for abnormal karyotype vs 76.7% for normal karyotype, P = .91), PNH clone at diagnosis (71.4% for PNH clone vs 77% for no PNH clone, P = .77), prior corticosteroid treatment (78.3% for prior corticosteroid treatment vs 76.1% for no prior corticosteroid treatment, P = .82), time from diagnosis to IST (79.2% for ≤ 14 days vs 73.3% for > 14 days, P = .35), and prior CSA treatment (76.9% for prior CSA treatment vs 73.1% for no prior CSA treatment, P = .67).

TABLE 3 Further treatment and response

Treatment, n (%)	p-ALG (n = 142)	r-ATG (n = 47)	Response at last follow-up
Eltrombopag	2 (1)	1 (2)	1 CR, 1 PR, 1 NR
A second course of IST	1 (1) (HD-CTX)	1 (2) (p-ALG)	1 NR, 1 CR
Transplanted	7 (5)	7 (15)	13 CR, 1NR
Haploidentical HSCT	3	7	10 CR
MUD-HSCT	3	0	2 CR, 1 NR
MSD-HSCT	1	0	1 CR

Abbreviation: MUD-HSCT, matched unrelated donor hematopoietic stem cell transplantation.

We then evaluated the response rates at 12 months in the p-ALG and r-ATG groups and found them to be equally distributed when patients were classified by ARC (at 1 month) and rhTPO treatment (data not shown).

3.3 | Further treatment

Further treatment and response at the last follow-up are shown in Table 3. Two (1.4%) patients in the p-ALG group and one (2.1%) patient in the r-ATG group received eltrombopag therapy. One (0.7%) patient in the p-ALG group received a second course of IST

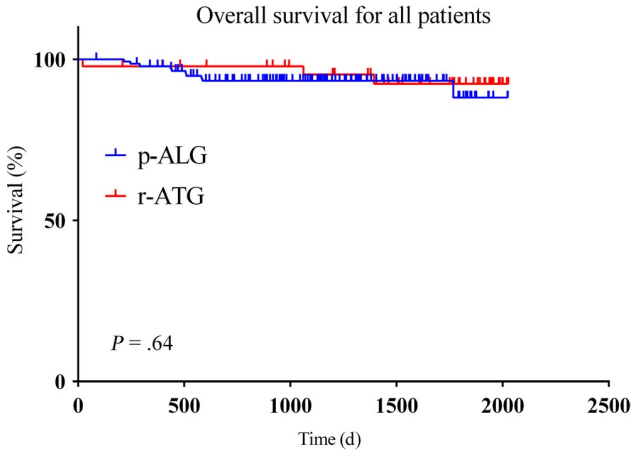

FIGURE 2 Overall survival of patients receiving porcine anti-lymphocyte immunoglobulin (p-ALG)- vs rabbit anti-thymocyte immunoglobulin (r-ATG)-based IST

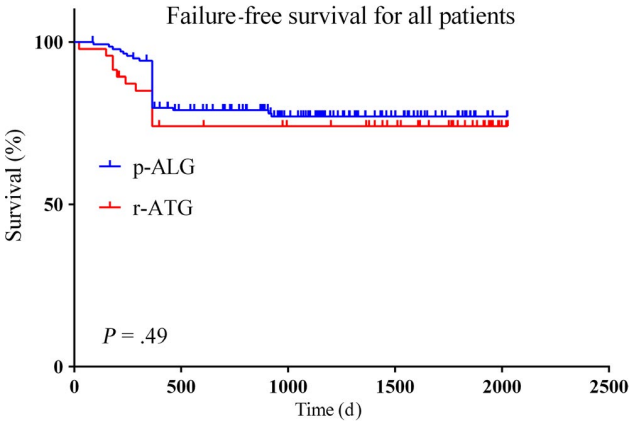

FIGURE 3 Failure-free survival of patients receiving porcine anti-lymphocyte immunoglobulin (p-ALG)- vs rabbit anti-thymocyte immunoglobulin (r-ATG)-based immunosuppressive therapy

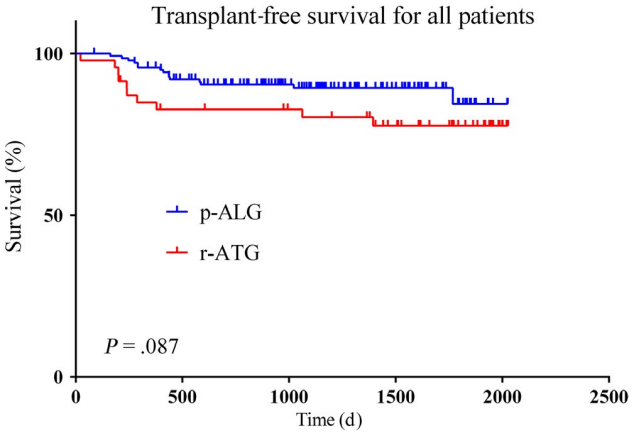

FIGURE 4 Transplant-free survival of patients receiving porcine anti-lymphocyte immunoglobulin (p-ALG)- vs rabbit anti-thymocyte immunoglobulin (r-ATG)-based immunosuppressive therapy

with high-dose cyclophosphamide. One (2.1%) patient in the r-ATG group received a second course of IST with p-ALG. Fourteen (7.4%) patients received allogeneic HSCT (ten haploidentical HSCT, three matched unrelated donor HSCT, one matched sibling donor HSCT), seven (4.9%) in the p-ALG group and seven (16.7%) in the r-ATG group (P = .05), and the median time from IST to transplantation was 282 (range 162-1022) days.

3.4 | Early death, recurrence, and clonal evolution

During the follow-up, only one (0.5%) early death was observed in the two groups. Seven (3.7%) patients relapsed, six (4.2%) in the p-ALG group and one (2.1%) in the r-ATG group (P = .83). The median time from IST to relapse was 720 (range, 230-1508) days. Three patients developed a new PHN clone, and all were in the p-ALG group (2.1%); two (15.4%) patients in the p-ALG group with a PHN clone developed symptomatic PNH. One patient in each of the p-ALG (0.7%, 305 days) and r-ATG groups (2.4%, 169 days) developed myelodysplastic syndrome after IST treatment.

3.5 | Survival and mortality

We compared the 5-year OS after IST and found no significant difference between the two groups (88% for p-ALG group vs 92% for r-ATG group, P = .64; Figure 2). No significant difference was observed in 5-year FFS between the p-ALG and r-ATG groups (65% for p-ALG group vs 63% for r-ATG group, P = .49; Figure 3). No significant difference was observed in 5-year TFS between the p-ALG and r-ATG groups (84% for p-ALG group vs 78% for r-ATG group, P = .087) (Figure 4).

We performed a univariate analysis for FFS and OS in term of sex, age, disease severity, abnormal cytogenetic clone, disease etiology, prior CSA treatment, time of prior CSA treatment, prior corticosteroid treatment, rhTPO treatment, disease duration, time from diagnosis to IST, complete blood count at disease diagnosis, and ARC 1 month after IST with the same cutoff as the response section (median of variables to be analyzed). We found that disease severity, rhTPO treatment, prior CSA treatment, neutrophil count (NEU), and ART at disease diagnosis significantly affected OS. However, only rhTPO treatment and prior CSA treatment significantly affected OS in the multivariate analysis (Table 4). We also performed the same univariate analysis on FFS and found that NEU at disease diagnosis and ART at disease diagnosis and 1 month significantly affected TFS. In addition, there tended to be a significant difference in FFS in terms of the rhTPO treatment. A multivariate analysis revealed that ART at 1 month after IST significantly affected FFS (Table 5).

During the follow-up period, there were 13 (6.9%) deaths in the two groups, 10 (7%) in the p-ALG group and 3 (7.1%) in the r-ATG group (P = .86). Twelve of the 13 patients died of complications of disease progression (six died of infection, three died of hemorrhage, one died of infection and hemorrhage, two died of heart failure), and only one patient died of graft-vs-host disease from allo-HSCT.

TABLE 4 Univariate and multivariate analyses for overall survival (OS)

		Univariate analysis		Multivariate analysis	
Variables	**Subtype**	**Mean OS (days)**	***P*-value**	**HR**	***P*-value**
Disease severity	VSAA	1648	.02	1.55	.48
	SAA	1825			
	NSAA	1981			
NEU at disease diagnosis	NEU ≤ 0.5×10^9/L	1860	.038	0.3	.2
	NEU > 0.5×10^9/L	1987			
ART at disease diagnosis	ARC ≤ 15×10^9/L	1830	.013	0.25	.13
	ARC > 15×10^9/L	1981			
rhTPO treatment	TPO treatment	1973	.006	5.8	.004
	No TPO treatment	1797			
Prior CSA treatment	Prior CSA treatment	1949	.005	5.9	.004
	No prior CSA treatment	1640			

Abbreviation: HR, hazard ratio.

TABLE 5 Univariate and multivariate analyses for failure-free survival

		Univariate analysis		Multivariate analysis	
Variables	**Subtype**	**Mean FFS (days)**	***P*-value**	**HR**	***P*-value**
NEU at disease diagnosis	NEU ≤ 0.5×10^9/L	1531	.031	0.66	.33
	NEU > 0.5×10^9/L	1768			
ART at disease diagnosis	ARC ≤ 15×10^9/L	1495	.031	1.05	.91
	ARC > 15×10^9/L	1735			
ART at 1 mo	ARC ≤ 40×10^9/L	1493	.003	0.39	.015
	ARC > 40×10^9/L	1809			
rhTPO treatment	TPO treatment	1709	.05	1.74	.092
	No TPO treatment	1484			

Abbreviation: HR, hazard ratio.

3.6 | Kinetics of lymphocyte depletion

Absolute lymphocyte counts are shown in Figure 5, and no significant difference was observed between the p-ALG and r-ATG groups before the IST treatment. However, there were significant differences between the two groups on days 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and at week 2, week 3, and 1 month after the IST treatment ($P < .05$).

3.7 | Adverse events and toxicity

The incidence of adverse events is shown in Table 6. No significant differences were observed between the p-ALG and r-ATG groups for most of the events, except fever and rigor, which were more prevalent in the r-ATG group ($P = .000$ and $P = .000$). Only one treatment-related death occurred due to severe infection.

3.8 | Economic analysis

The median first-time hospital stays were 87 (range, 38-202) days for the p-ALG group and 97 (range, 40-200) days for the r-ATG group ($P = .089$). The median first-time hospitalization expenses were 181 958 (range, 79 563-771 558) yuan in the p-ALG group and 246 397 (range, 108 292-436 485) yuan in the r-ATG group ($P = .000$).

4 | DISCUSSION

Immunosuppressive therapy with ATG and CSA is an effective front-line treatment for SAA and transfusion-dependent NSAA when there is no histocompatible sibling donor for children and young adults or the patient's age is >35-50 years old.[3] The standard regimen for first-line IST remains ATG and CSA, which induces hematological

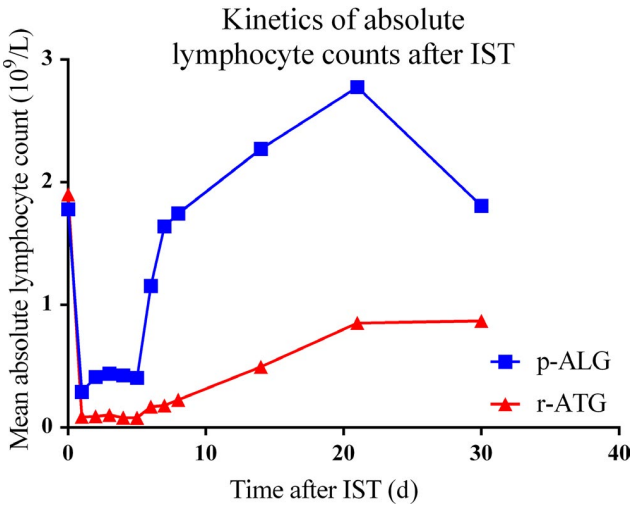

FIGURE 5 Mean absolute lymphocyte counts of patients receiving porcine anti-lymphocyte immunoglobulin (p-ALG)- vs rabbit anti-thymocyte immunoglobulin (r-ATG)-based immunosuppressive therapy

recovery in 50-70% of cases with excellent long-term survival among responders.[8,12-15] Some studies have reported that children tend to have a higher response rate.[16-18] In this retrospective study, we analyzed the response rates, treatment-related toxicity, and survival of 189 children with AA treated with p-ALG- or r-ATG-based IST. The majority of the patients had long-term follow-ups. This study provides strong evidence supporting the use of p-ALG in children with AA.

The response rates and OS in children with AA treated with p-ALG or r-ATG and CSA in our study were better than those reported by other studies,[6,17,19] which may be related to ethnicity, inclusion of transfusion-dependent NSAA, longer follow-up time, and a stronger CSA tapering strategy for our patients. In our experience, CSA was administered until the best response was achieved. Then, it was held for 3 months and followed by a slow taper until discontinuation. If CSA dependence developed, then the hold on the CSA lasted until the final follow-up. Rather than administering CSA for a given time,[13,19] our CSA tapering strategy induced a lower relapse rate leading to higher survival. Although r-ATG induced more prolonged and profound lymphopenia than p-ALG, no significant difference in response rates was observed between the two groups at 3, 6, 12, or 24 months, indicating that p-ALG and r-ATG have similar therapeutic efficacy. We hypothesized that the lower strength of p-ALG immunosuppression compared to that of r-ATG was sufficient to protect against autoimmune-mediated abnormal immunity. This may also induce fewer infections, such as Epstein-Barr virus and reactivation of cytomegalovirus.

Some studies have reported that children with VSAA have a higher complete response rate and better survival compared to children with SAA,[17] while others claim no significant differences between the two types of patients.[19] However, we found both higher complete responses at 6 and 12 months after treatment and better OS for SAA compared to VSAA patients treated with p-ALG- or r-ATG-based IST in our study. This can be explained by the fewer residual hematopoietic stem cells in VSAA patients than in SAA patients, which led to more difficulties or a longer time to achieve successful hematopoietic reconstitution. In our study, a significant difference was detected in the time of acquired response after IST between the VSAA (51, range 3-469 days) and SAA (37,

TABLE 6 Adverse events and toxicity after porcine anti-lymphocyte immunoglobulin (p-ALG) and rabbit anti-thymocyte immunoglobulin (r-ATG)

Adverse events, n (%)	No. of events after p-ALG	No. of events after r-ATG	*P*-value
Infection	81 (57)	32 (68)	NS
Upper respiratory tract infection	36 (25)	17 (40)	NS
Fever of unknown origin	28 (20)	8 (17%)	NS
Pulmonary infection	5 (4)	3 (6)	NS
Skin and soft-tissue infection	6 (4)	2 (4)	NS
Sepsis	4 (3)	2 (4)	NS
Oral cavity infection	5 (4)	0	NS
Urinary tract infection	1 (1)	1 (2)	NS
Perianal infection	2 (1)	0	NS
Septic shock	2 (1)	1 (2)	NS
Others	6 (4)	4 (9)	NS
Hemorrhage	13 (9)	4 (9)	NS
Elevated liver-function tests	8 (6)	5 (11)	NS
Median ALT, U/L (range)	172 (68-733)	131 (120-303)	ND
Median AST, U/L (range)	111 (30-973)	222 (39-261)	ND
Renal impairment	3 (2)	2 (4)	NS
Fever	27 (19)	29 (62)	0.000
Rigor	5 (4)	10 (21)	0.000
Rash	56 (39)	16 (34)	NS
Pruritus	31 (22)	9 (19)	NS
Serum sickness	68 (48)	24 (51)	NS
Arthralgia	40 (28)	15 (32)	NS
Myalgia	32 (23)	12 (26)	NS
Others			
Mouth ulcers	23 (16)	13 (28)	NS
Gastrointestinal reaction	9 (6)	5 (11)	NS
Hypertension	10 (7)	2 (4)	NS
Fluid retention	7 (5)	0	NS

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

range 2-495 days) groups (P = .007). Furthermore, there was a significant difference in ART at 1 month after IST between the VSAA (18.6×10^9/L, range 2.1-199) and SAA (47.3×10^9/L, range 3.4-155.5) groups (P = .000). All of these data support the notion that SAA patients have a better response and survival compared to VSAA patients after IST treatment.

We also identified rhTPO treatment and prior CSA treatment as significant variables for OS in our multivariate survival analyses. Recombinant human c-MPL ligand (thrombopoietin) acts on megakaryocyte progenitors as well as on erythroid and multipotential progenitors in vitro.[20] In our experience, administering rhTPO improves the hematological response and bone marrow recovery as well as reducing the need for transfusion in AA patients receiving IST. One study reported long-term improvement of AA in patients after short-term administration of rhTPO.[21] Furthermore, rhTPO has been reported to have a therapeutic effect by promoting platelet engraftment and improving the prognosis of patients with AA after allo-HSCT.[22] These results imply a partial therapeutic role of rhTPO as adjuvant therapy in the treatment of AA. However, a recent study showed that proinflammatory cytokine interferon-γ (IFN-γ) specifically prevents full engagement of TPO to c-MPL via formation of TPO:IFN-γ heteromer, which lead to perturbation of TPO-induced signaling pathways and decreased survival of human hematopoietic stem and progenitor cells (HSPCs). Eltrombopag, a synthetic small molecule mimetic of TPO that interacts with c-MPL at a position distinct from the extracellular binding site of TPO, bypasses this inhibition.[23] This explains for its clinical activity in bone marrow failure, despite already elevated endogenous TPO levels. Even so, we speculated that high concentration and long-term treatment of TPO may overcome this perturbation by neutralize the IFN-γ in bone marrow microenvironment of AA patients, which may lead to long-term clinical effect. The good news is that we are now conducting a randomized controlled trial to evaluate the role of rhTPO in the treatment of AA in our center. We are expecting a positive result of this trial. After all, rhTPO have lower cost and more accessible compare to eltrombopag for Chinese patients with AA. Although we identified rhTPO as a prognostic factor, no difference was observed in usage of rhTPO between the p-ALG and r-ATG groups. It means there will be no significant impact on comparison of the efficacy and safety of the two groups resulted from use of rhTPO.

Although prior CSA treatment significantly affected survival in our multivariate survival analyses, no significant differences in OS were observed between different time intervals of prior CSA treatment (P = .89). Instead, we observed significantly more VSAA patients in the prior time of CSA treatment ≤14 days group than in its counterpart (42% vs 23%, P = .0065). This may be because most VSAA patients have a short disease duration and a higher probability of selecting alternative donor allo-HSCT rather than IST. In contrast, SAA patients have a longer disease duration and a higher probability of selecting IST. In this situation, more SAA patients in the prior CSA treatment group led to higher survival.

The major adverse events in both groups were infection, hemorrhage, and symptoms resulting from a transfusion reaction or p-ALG or r-ATG serum sickness. Although r-ATG seems to be more immunosuppressive than p-ALG, we observed a similar incidence of infection and hemorrhage in the two groups in the present study. Furthermore, no difference in first-time hospital stay was observed between the two groups, even though first-time hospitalization expenses were higher in the r-ATG group than in the p-ALG group. Other events included elevated liver-function tests and impaired renal function, both of which may have resulted from the administration of CSA. No significant difference was detected in the incidence of serum sickness between p-ALG and r-ATG. However, significant differences were observed for fever and rigor, and a further investigation revealed that the majority of the cases developed within the first 5 days of IST, indicating a greater frequency of transfusion reactions for r-ATG than p-ALG.

Our results imply that the therapeutic efficacy and safety of p-ALG combined with CSA do not differ significantly from those of r-ATG combined with CSA as first-line therapy for children with AA. Furthermore, p-ALG has the advantage of significantly lower cost than r-ATG.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ORCID

Fengkui Zhang https://orcid.org/0000-0002-2797-1339

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·短篇论著·

猪抗人淋巴细胞球蛋白与兔抗人胸腺细胞球蛋白治疗重型再生障碍性贫血儿童的疗效比较

孔令军 何海龙 胡绍燕
苏州大学附属儿童医院血液科 215003
通信作者:何海龙,Email:childhe@sina.com
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Comparison of porcine antilymphocyte globulin (p-ALG) and rabbit antithymocyte globulin (r-ATG) in the treatment of children with severe aplastic anemia

Kong Lingjun, He Hailong, Hu Shaoyan
Department of Hematology, Children's Hospital of Soochow University, Suzhou 215003, China
Corresponding author: He Hailong, Email: childhe@sina.com

儿童重型/极重型再生障碍性贫血(SAA/VSAA)比例高于成人,预后较差[1],allo-HSCT及免疫抑制治疗(IST)为其首选治疗方案[2]。本研究我们通过对单中心病例的回顾性分析,比较环孢素A(CsA)联合猪抗人淋巴细胞球蛋白(p-ALG)或兔抗人胸腺细胞球蛋白(r-ATG)治疗儿童SAA/VSAA的疗效及安全性。

病例与方法

1. 病例:回顾性分析2011年10月至2017年11月我院收治的诊断为SAA/VSAA且接受IST的166例患儿临床资料。诊断符合《儿童获得性再生障碍性贫血诊疗建议》[2],并经多参数嗜水气单胞菌溶素变异体(Flaer)检测、先天性遗传代谢基因筛查等,除外先天性AA及可致全血细胞减少的其他疾病。同时排除患儿毒物药物暴露史;排除免疫异常性疾病等。AA分型标准参照Camitta分型标准[3]。所有患儿IST前未行AA相关治疗,本研究经医院伦理委员会备案批准,其中接受p-ALG治疗的63例设为p-ALG组,接受r-ATG治疗的103例设为r-ATG组。

2. 治疗方法:IST:p-ALG(武汉生物制品研究所产品)25 mg·kg^{-1}·d^{-1}×5 d或r-ATG(法国赛达药厂产品)3.75 mg·kg^{-1}·d^{-1}×5 d,静脉滴注;CsA以5 mg·kg^{-1}·d^{-1}每12 h 1次起始,r-ATG/p-ALG输注结束后第1天开始,维持CsA血清谷浓度150~250 μg/L。支持治疗包括红细胞及血小板输注、G-CSF等。所有治疗在层流病房或净化帐中进行。

3. 疗效评估及不良反应评价标准:严格参照文献[2]疗效标准,完全缓解(CR)及部分缓解(PR)为有效,未缓解、死亡或转化为无效(NR)。判定疗效时需至少3个月内未接受成分输血或G-CSF治疗。转化的评判标准为治疗后通过其他检查证实患儿疾病有变化,不再达到AA的诊断标准;复发的评判标准为脱离血制品输注3个月后需再次输注。比较患儿治疗期间及治疗后不良反应:感染、r-ATG/p-ALG使用时类过敏反应、输注血小板及使用后血清病样反应。评价标准为:患儿排除治疗前感染因素,至应用p-ALG或r-ATG后1个月内,在治疗中及治疗后新出现血培养、痰培养等阳性指标,影像学表现阳性及临床体格检查显示存在感染,诊断为脓毒血症、重症肺炎定义为患儿严重事件;患儿r-ATG/p-ALG用药时排除感染的发热、皮疹、呛咳、关节肿痛等为类过敏反应,因此停药或者死亡者定义为严重事件;用药7~10 d后出现发热、皮疹、关节肿痛、肺部渗出、骨质损害等为血清病样反应,骨质损害定义为严重事件。

4. 随访:随访截至2019年5月1日,r-ATG组中位随访25(16~79)个月,p-ALG组中位随访38(16~81)个月,观察指标包括:患儿疾病状态、治疗时及治疗后发生类过敏反应、血清病样反应情况,血常规,原发病是否有复发、进展、转化,患儿是否规律用药,患儿死亡原因及死亡时间。无进展生存(PFS)时间定义为患儿从接受治疗开始至疾病进展或死亡的时间。

5. 统计学处理:采用SPSS 24.0进行统计学分析。连续变量采用均值±标准差或M(P25,P75)进行描述,组间比较采用t检验或Mann-Whitney U检验;分类变量组间比较采用χ^2检验或Fisher精确概率法。生存分析采用Kaplan-Meier法,组间比较采用Log-rank检验。$P<0.05$为差异有统计学意义。

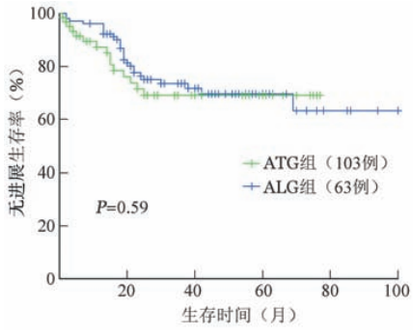
结 果

1. 患儿基本特征:r-ATG组与p-ALG组患儿临床特征比较见表1,除r-ATG组初诊时WBC高于p-ALG组[(3.46±0.23)×10^9/L对(2.78±0.13)×10^9/L,$t=2.74$,$P=0.012$],两组年龄、性别、骨髓增生程度(增生低下/增生重度低下)、有无合并感染、疾病类型(SAA/VSAA)、初诊时HGB、PLT、ANC

差异均无统计学意义。

2. 疗效比较：随访至2019年5月，至随访终点166例患儿总有效率（ORR）为74.1%，其中CR 75例（45.2%）、PR 48例（28.9%）。r-ATG组ORR为72.8%（75/103），其中CR 44例（42.7%）、PR 31例（30.0%）；p-ALG组ORR为76.2%（48/63），其中CR 31例（49.2%），PR 17例（27.0%），两组ORR及CR率差异均无统计学意义（χ^2分别为0.23、0.66，P值分别为0.63、0.41）。

3. 生存分析：r-ATG组与p-ALG组5年PFS率分别为（73.5±4.7）%、（69.1±6.8）%，差异无统计学意义（$\chi^2=0.291$，$P=0.59$）（图1）。



r-ATG：兔抗人胸腺细胞球蛋白；p-ALG：猪抗人淋巴细胞球蛋白

图1 r-ATG组与p-ALG组重型/极重型再生障碍性贫血患儿无进展生存曲线

4. 治疗后外周血WBC变化：r-ATG组与p-ALG组治疗后3、6、9个月时WBC差异均有统计学意义（P值均<0.05）；治疗后12个月时WBC差异无统计学意义（$t=1.460$，$P=0.094$）（表2）。

5. 相关不良反应及输血情况比较：166例患儿治疗后82例（49.4%）发生感染，r-ATG组感染发生率为57.3%（59/103），p-ALG组感染发生率为36.5%（23/63），差异有统计学意义（$\chi^2=6.749$，$P=0.009$），两组感染严重事件发生率分别为20.4%（21/103）对20.6%（13/63），差异无统计学意义（$\chi^2=0.001$，$P=0.970$）。

表2 r-ATG组与p-ALG组重型/极重型再生障碍性贫血患儿治疗后不同时间外周血WBC比较（×10⁹/L，$\bar{x}\pm s$）

组别	例数	3个月	6个月	9个月	12个月
r-ATG组	103	3.23±0.23	3.37±0.14	3.83±0.16	4.36±0.17
p-ALG组	63	4.24±0.22	4.24±0.26	4.50±0.30	4.76±4.37
t值		−2.47	−3.11	−2.13	−1.46
P值		<0.001	0.009	0.043	0.094

注：r-ATG：兔抗人胸腺细胞球蛋白；p-ALG：猪抗人淋巴细胞球蛋白

r-ATG与p-ALG药物应用时主要不良反应为类过敏反应，其发生率r-ATG组为50.5%（52/103），p-ALG组为33.3%（21/63），差异有统计学意义（$\chi^2=4.668$，$P=0.031$），其中严重事件发生率分别为1.9%（2/103）、6.3%（13/63），差异有统计学意义（$P<0.001$）。

103例r-ATG组患儿中85例（82.5%）需要血小板输注支持，63例p-ALG组患儿中46例（73.0%）接受血小板输注，差异无统计学意义（$\chi^2=2.124$，$P=0.145$）。

r-ATG组与p-ALG组血清病样反应发生率分别为62.1%（64/103）、60.3%（38/63），其中严重事件发生率分别为3.9%（4/103）、4.8%（3/63），差异均无统计学意义（P值分别为0.815、1.000）。

6. 转化及死亡情况比较：随访6个月，2例患儿发生转化，均为r-ATG组患儿，分别于ATG/ALG治疗后第94、136天诊断为骨髓增生异常综合征。至随访终点死亡2例，均为r-ATG组患者，1例为r-ATG治疗第3天出现肌肉疼痛，下肢肿胀，随之波及全身，诊断为类过敏反应导致的渗漏综合征，出现血压下降等弥散性血管内凝血（DIC）表现，抢救无效而死亡；另1例为r-ATG治疗后第146天因为脓毒血症、血小板低并发颅内出血死亡。

表1 r-ATG组和p-ALG组重型/极重型再生障碍性贫血（SAA/VSAA）患儿基本特征比较

项目	r-ATG组（103例）	p-ALG组（63例）	统计量	P值
中位年龄（岁）	7（2～11）	7（1～15）	−1.60	0.135
性别（例，男/女）	53/50	35/28	0.26	0.607
骨髓增生程度[例（%）]			0.03	0.873
增生低下	52（50.5）	31（49.2）		
增生重度低下	51（49.5）	32（50.8）		
合并感染[例（%）]	16（15.5）	10（15.9）	<0.01	0.953
疾病类型[例（%）]			<0.01	0.960
SAA	56（54.4）	34（54.0）		
VSAA	47（45.6）	29（46.0）		
初诊时血常规				
WBC（×10⁹/L，$\bar{x}\pm s$）	3.46±0.23	2.78±0.13	2.74	0.012
HGB（g/L，$\bar{x}\pm s$）	72.01±1.94	72.27±2.68	1.73	0.850
PLT[×10⁹/L，M（$P25$，$P75$）]	20（8～25）	23（11～29）	−2.23	0.056
ANC[×10⁹/L，M（$P25$，$P75$）]	0.67（0.27～0.14）	0.57（0.21～1.03）	1.18	0.101

注：r-ATG：兔抗人胸腺细胞球蛋白；p-ALG：猪抗人淋巴细胞球蛋白

讨 论

T细胞介导的免疫机制异常和骨髓造血功能损伤被公认为获得性AA发病的主要原因[1]。IST的作用机制为干扰机体自身异常的免疫功能,从而保存残存的造血功能,其中r-ATG和p-ALG治疗原理为通过其可显著清除正常人外周血淋巴细胞特性起作用[4]。无论使用哪种药物,最终治疗目的是恢复骨髓造血功能[5]。但治疗中r-ATG和p-ALG的选择可能存在疑问,临床医师对于种属剂型的决策可能影响到患儿的预后。基于上述原因,比较r-ATG和p-ALG在AA临床治疗中的疗效具有重要意义。

本组病例r-ATG组ORR为72.8%,5年PFS率为(73.5±4.7)%,与C1é等[6]研究相似,较日本成人比例高[7];p-ALG组ORR为76.2%,5年PFS率为(69.1±6.8)%,与国内Chen等[8]报道一致;组间比较表明,ORR及5年PFS率差异无统计学意义,与国内Liu等[9]研究结果相近。本研究中监测r-ATG组和p-ALG组治疗后不同时间的血常规时发现,在初诊时及p-ALG/r-ATG治疗后3、6、9个月时外周血WBC差异有统计学意义,初诊r-ATG组WBC高于p-ALG组,但治疗后p-ALG组较r-ATG组恢复快,由此我们初步推论r-ATG早期对淋巴细胞尤其是对调节性T细胞的杀伤力可能较p-ALG强,但这需要进一步的临床及实验室检查论证。

感染是SAA/VSAA患儿治疗期间主要并发症,也是导致严重不良后果的主要因素之一[10],本研究2例死亡患儿中1例为感染导致。本研究中IST后感染总发生率为49.4%,与国内陶晓虹及董红华[11]报道相似,其中r-ATG组感染发生率高于p-ALG组,这可能与r-ATG组治疗白细胞恢复慢有关系;但感染严重事件如脓毒血症、重症肺炎发生率两组差异无统计学意义,可能与SAA/VSAA患儿整体均存在免疫缺陷有关。目前针对AA患儿预防性应用抗生素的安全性和有效性的对照研究报道较少,但《再生障碍性贫血诊断与治疗中国专家共识(2017年版)》[5]指出AA患者发热应按《中国中性粒细胞缺乏伴发热患者抗菌药物临床应用指南(2016年版)》[12]治疗原则来处理;同时结合《血液病/恶性肿瘤患者侵袭性真菌病的诊断标准与治疗原则(第五次修订版)》[13],当AA患者治疗后ANC<0.5×10^9/L时,需预防性口服抗真菌药物;当出现粒细胞缺乏时,发热超过1 h或血象恢复后感染超过48 h应该应用抗菌药物;当感染超过96 h并具有影像学和临床表现者,应进行抗真菌治疗。同时SAA/VSAA患儿治疗过程中需重视IST后类过敏反应及血清病样反应,本研究中r-ATG组类过敏反应发生率为50.5%,高于国内苏雁等[14]报道及p-ALG组,且1例患儿死于过敏反应导致的渗漏综合征,但严重反应发生率低于p-ALG组。而两组血清病样反应发生率差异无统计学意义,与刘苏等[15]报道基本一致。

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CsA 联合 ATG 或 ALG 治疗儿童 SAA 的疗效分析

杨卫东[1]，刑海洲[2]
（1. 河南省濮阳市安阳地区医院 血液二科，河南 安阳 455000；2. 郑州大学第一附属医院 血液科，河南 郑州 450052）

摘要：目的 比较环孢素 A（CsA）联合生物种属免疫抑制剂抗人胸腺细胞球蛋白（ATG）与淋巴细胞球蛋白（ALG）治疗儿童重型再生障碍性贫血（SAA）的疗效及安全性。**方法** 选取 2010 年 4 月 -2016 年 8 月于河南省濮阳市安阳地区医院收治的 93 例 SAA 患儿，根据治疗方案分组，对两组患儿的疗效进行比较分析。**结果** 治疗 3、6、9 及 12 个月时，r-ATG 组的 OR 分别为 58.54%、73.17%、80.49% 和 80.49%；p-ALG 组的 OR 分别为 46.15%、75.00%、75.00% 和 76.92%，组间比较无差异（$P>0.05$）；在应用 r-ATG 或 p-ALG 过程中，r-ATG 组应用时过敏所致发热患儿数高于 p-ALG 组（$P<0.05$），r-ATG 组过敏反应总发生率高于 p-ALG 组（$P<0.05$）；在应用 r-ATG 或 p-ALG 后，r-ATG 组与 p-ALG 组的血清病总发生率分别为 60.98% 与 61.54%，感染总发生率分别为 34.15% 与 32.69%，组间比较无差异（$P>0.05$）；r-ATG 组与 p-ALG 组的总有效率比较无差异（$P>0.05$）。**结论** CsA 分别联合 r-ATG 或 p-ALG 治疗儿童 SAA 在缓解血液学反应方面效果相当，治疗过程中 p-ALG 较 r-ATG 在诱发过敏反应方面风险更低。

关键词： 重型再生障碍性贫血；儿童；兔抗人胸腺细胞球蛋白；猪抗人淋巴细胞球蛋白
中图分类号： R332　　**文献标识码：** A

Comparative analysis of CsA combined with immunosuppressive agents of different species in treatment of children with SAA

Wei-dong Yang[1], Hai-zhou Xing[2]
(1. Department of Hematology, Anyang District Hospital, Anyang, Henan 455000, China; 2. Department of Hematology; the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China)

Abstract: Objective To compare the efficacy and safety of cyclosporine A (CsA) combined with immunosuppressive agents of different species in the treatment of children with severe aplastic anemia (SAA). **Methods** From April 2010 to August 2016, 93 cases of SAA children admitted to Anyang District Hospital were grouped according to the treatment plan. The patients in the r-ATG group (41 patients) received CsA combined with r-ATG and those in the p-ALG group (52 patients) received CsA combined with p-ALG. The treatment effect was compared between the two groups. **Results** In 3, 6, 9 and 12 months of treatment, the OR of the r-ATG group was 58.54%, 73.17%, 80.49% and 80.49% respectively, the OR of the p-ALG group was 46.15%, 75.00%, 75.00% and 76.92%, there were no significant differences between the two groups ($P>0.05$). In the application of r-ATG or p-ALG, the number of children with anaphylaxis fever in the r-ATG group was significantly larger than that in the p-ALG group ($P<0.05$), the total incidence of hypersensitivity in the r-ATG group was significantly higher than that in the p-ALG group

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($P < 0.05$). After treatment with r-ATG or p-ALG, the total incidence of serum disease in the r-ATG group and the p-ALG group was 60.98% and 61.54% respectively ($P > 0.05$), the total incidence of infection was 34.15% and 32.69% respectively ($P > 0.05$), there was no significant difference in the overall effective rate between the two groups ($P > 0.05$). **Conclusions** CsA combined with r-ATG or p-ALG has significant effect in relieving hematologic response for treatment of children with SAA. During treatment, p-ALG has a significantly lower risk in the induction of allergic reactions than r-ATG.

Keywords: SAA; children; r-ATG; p-ALG

再生障碍性贫血（aplastic anemia, AA）是一种多种原因导致的骨髓造血功能衰竭性疾病，临床表现为进行性贫血、出血及反复感染，已成为严重危害儿童健康的主要血液病之一[1]。重型 AA（severe aplastica anemia, SAA）具有起病急与病情进展迅猛的特点，通常需依赖输血维持生命，同时还合并有并发症，临床病死率较高，治疗难度颇大。抗人胸腺细胞球蛋白 / 淋巴细胞球蛋白（anti-thymocyte globulin/anti-lymphocyte globulin, ATG/ALG）联合环孢素 A（Cyclosporin A, CsA）的免疫抑制疗法（immunosuppressive therapy, IST）是当前临床治疗 AA 的主要手段。根据免疫动物种属的不同，ATG/ALG 目前包括有马抗人淋巴细胞球蛋白（horse antihuman lymphocyte globulin, h-ALG）、兔抗人胸腺细胞球蛋白（rabbit antihuman thymocyte globulins, r-ATG）及猪抗人淋巴细胞球蛋白（pig antihuman lymphocyte globulin, p-ALG）等，后两种在国内应用广泛，但何为优选种属尚无定论[2-6]。鉴于此，本研究对比了 CsA 联合 r-ATG 或 p-ALG 治疗 SAA 的有效性与安全性。

1 资料与方法

1.1 一般资料

选取 2010 年 4 月 -2016 年 8 月于河南省濮阳市安阳地区医院收治的 93 例 SAA 患儿。纳入标准：①SAA 诊断与分型均符合相关标准[7]；②年龄 2 ~ 16 岁，性别不限；③获得医院医学专业学术委员会和伦理委员会批准，患儿家属签署知情同意书。排除标准：①合并其他免疫疾病、特殊感染及毒物药物暴露史者；②经彗星及丝裂霉素实验证实为先天性骨髓衰竭，经骨髓细胞形态学检查及细胞遗传学检查证实为骨髓增生异常综合征，经流式细胞仪检测阵发性睡眠型血红蛋白尿克隆 <50%；③本次治疗前已接受过异基因造血干细胞或 IST 治疗者；④对本次治疗方案用药具过敏体质者。接受 CsA 联合 r-ATG 治疗 41 例患儿作为 r-ATG 组，接受 CsA 联合 p-ALG 治疗 52 例患儿作为 p-ALG 组。两组患者治疗前一般资料比较，差异无统计学意义（P >0.05）。见表 1。

1.2 方法

1.2.1 联合 IST 治疗 两组患儿在正式治疗前均接受感染情况及肝肾功能检查。治疗场所选择相对隔离的净化帐或清洁病房。r-ATG 组采用 CsA（杭州中美华东制药有限公司，国药准字 H10960121）联合 r-ATG（北京北生研生物制品有限公司，国药准字 S10870083）治疗，p-ALG 组采用 CsA 联合 p-ALG（武汉生物制品研究所有限责任公司，国药准

表 1 两组患儿治疗前的临床特征比较

组别	男 / 女 / 例	年龄 /（岁，$\bar{x} \pm s$）	SAA 分型（SAA-Ⅰ / SAA-Ⅱ）/ 例	诊治间隔时间（≤ 30 d/> 30 d）/ 例	血常规			
					WBC（$\times 10^9$/L，$\bar{x} \pm s$）	Hb（g/L，$\bar{x} \pm s$）	Plt[$\times 10^9$/L，M（P_{25}，P_{75}）]	ANC[$\times 10^9$/L，M（P_{25}，P_{75}）]
r-ATG 组（n =41）	22/19	6.71 ± 0.98	35/6	29/12	3.12 ± 1.43	72.96 ± 17.43	13.3（9.05，15.5）	0.46（0.42，0.48）
p-ALG 组（n =52）	29/23	6.73 ± 1.10	45/7	42/10	3.53 ± 1.87	71.45 ± 20.23	13.6（11.2，14.9）	0.48（0.41，0.54）
$\chi^2/t/Z$ 值	0.041	-0.122	0.026	1.279	-1.566	0.458	-0.319	-1.604
P 值	0.839	0.904	0.871	0.258	0.121	0.648	0.75	0.109

字 S10830001）治疗。r-ATG 与 p-ALG 的治疗剂量分别为 25 mg/（kg·d）和 3.75 mg/（kg·d），均连续静脉给药 5 d。为最大程度避免严重不良反应的发生，两组患儿均在给药首日先以拟定剂量的 10% 配 100 ml 生理盐水行静脉滴注 1 h，若期间未观察到严重全身反应再增加至拟定给药剂量，全日总剂量需在 12 ~ 18 h 维持滴注。自治疗首日始，两组患儿均同步口服 CsA，剂量为 5 mg/（kg·d），在全天内以 12 h 为间隔均分为 2 次服用。治疗全程密切监测 CsA 血药浓度，以维持在 150 ~ 250 ng/ml 水平为宜，个别难以调整者应≥ 100 ng/ml。观察到患儿血液学指标缓解后继续巩固 6 个月再视情况停用 CsA。

1.2.2　辅助与支持治疗　两组患儿均在应用 r-ATG 或 p-ALG 时另开一条静脉通道滴注甲泼尼龙（焦作国药集团容生制药有限公司，国药准字 H20030727）以防止过敏反应，给药剂量为 2 mg/（kg·d），其中一半剂量在滴注 r-ATG 或 p-ALG 前 30 min 给予，另一半剂量行同步维持。r-ATG 或 p-ALG 给药结束后为防止血清病反应，两组患儿需勿停用甲泼尼龙，改剂量为 1 mg/（kg·d）静滴或口服，在 3 周内逐渐减停。r-ATG 或 p-ALG 用药结束后每 2 ~ 3 d 查血常规，若观察到感染中性粒细胞 < 0.5×10^9/L 需及时采用重组人粒细胞刺激因子行皮下注射，恢复中性粒细胞至≥ 1.0×10^9/L，另通过输血将血小板和血红蛋白分别维持在≥ 20×10^9/L 和 60g/L 左右水平。必要时合理应用抗生素以对抗感染，同时积极预防霉菌感染。

1.2.3　随访　本研究随访截止时间为 2017 年 2 月，随访形式为门诊收集患儿血常规，部分患儿通过电话随访病情变化，r-ATG 组和 p-ALG 组的中位随访时间分别为 47 个月（1 ~ 80 个月）和 51 个月（1 ~ 82 个月）。随访时统计复发及克隆转化情况，复发定义为脱离血制品输注 3 个月后需再次输注支持，克隆转化参考 SA 等人研究进行判断[8]。

1.3　效果评价

1.3.1　血液学反应评估　完全缓解（complete response, CR）：ANC>1.5×10^9/L，Hb>120 g/L，Plt>100×10^9/L，患儿可脱离输血支持；部分缓解（partial response,PR）：ANC>0.5×10^9/L，Hb>80 g/L，Plt>100×10^9/L，患儿已达不到 SAA 标准，可脱离输血支持；无效（none response, NR）：血象未见恢复迹象，仍必须接受输血支持[9]。总有效率（overall responserate,OR）=（CR+PR）/ 总例数 ×100%。分别于治疗 3、6、9 及 12 个月对患儿的血液学反应进行阶段性评估。

1.3.2　毒性反应与副作用　在 r-ATG 或 p-ALG 用药期间及使用后短期内（1 个月），观察并统计两组患儿的毒性反应及其副作用，包括 r-ATG 或 p-ALG 应用过程中的过敏反应与治疗后的血清病反应和感染情况。

1.4　统计学方法

数据分析采用 SPSS 19.0 统计学软件，计量资料以均数 ± 标准差（$\bar{x} \pm s$）或中位数和四分位间距 M（P_{25}，P_{75}）表示，比较采用 t 检验，等级资料以等级表示，采用秩和检验，计数资料以构成比或率（%）表示，比较采用 χ^2 或 Fisher 检验，用 Kaplan-Meier 法绘制生存曲线，P <0.05 为差异有统计学意义。

2　结果

2.1　两组患儿的血液学反应评估情况比较

治疗 3、6、9 及 12 个月时 r-ATG 组的 OR 分别为 58.54%、73.17%、80.49% 和 80.49%，p-ALG 组的 OR 分别为 46.15%、75.00%、75.00% 和 76.92%。两组患儿的血液学反应比较，差异无统计学意义（P >0.05）。见表 2。

2.2　两组患儿的毒性反应与副作用比较

两组患儿用药时过敏反应中的发热情况比较，差异有统计学意义（P <0.05），r-ATG 组患儿过敏反应总发生率为 53.66%，p-ALG 组患儿为 32.69%，两组比较，差异有统计学意义（P <0.05）；在应用 r-ATG 或 p-ALG 后，r-ATG 组与 p-ALG 组的血清病总发生率分别为 60.98% 与 61.54%，两组比较，差异无统计学意义（P >0.05），两组患儿感染总发生率分别为 34.15% 与 32.69%，两组比较，差异无统计学意义（P >0.05）。见表 3。

2.3　两组患儿治疗后 OS 比较

r-ATG 组与 p-ALG 组治疗 2 年后 OS 分别为 87.80% 与 84.62%；3 年后 OS 分别为 87.80% 与 78.85%；5 年后 OS 分别为 60.98% 与 53.85%，组间比较，差异均无统计学意义（P >0.05）。见附图。

表 2　两组患儿血液学反应评估情况比较　例（%）

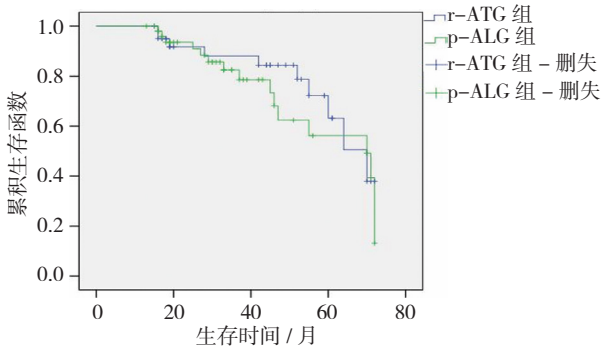
组别	CR	PR	NR	OR
r–ATG 组（n =41）				
3 个月	4（9.76）	20（48.78）	17（41.46）	24（58.54）
6 个月	12（29.27）	18（43.90）	11（26.83）	30（73.17）
9 个月	12（29.27）	21（51.22）	8（19.51）	33（80.49）
12 个月	21（51.22）	12（29.27）	8（19.51）	33（80.49）
p–ALG 组（n =52）				
3 个月	4（7.69）	20（38.46）	28（53.85）	24（46.15）
6 个月	9（17.31）	30（57.69）	13（25.00）	39（75.00）
9 个月	15（28.85）	24（46.15）	13（25.00）	39（75.00）
12 个月	30（57.69）	10（19.23）	12（23.08）	40（76.92）

表 3　两组患儿的毒性反应与副作用比较　例（%）

组别	过敏反应						
	发热	皮疹、瘙痒	呛咳	关节肿痛	头痛	终止病例数	合计
r–ATG 组（n =41）	13（31.71）	13（31.71）	1（2.44）	0（0.00）	1（2.44）	1（2.44）	22（53.66）
p–ALG 组（n =52）	5（9.62）	13（25.00）	2（3.85）	1（1.92）	0（0.00）	4（7.69）	17（32.69）
χ^2 值	7.168	0.512	0.044	–	–	0.425	4.139
P 值	0.007	0.474	0.834	1.000	0.441	0.514	0.042

组别	血清病							
	发热	皮疹、瘙痒	关节肿痛	胸、肺部渗出	腹、盆腔积液	头痛	黏膜损害	合计
r–ATG 组（n =41）	12（29.27）	14（34.15）	1（2.44）	1（2.44）	0（0.00）	1（2.44）	1（2.44）	25（60.98）
p–ALG 组（n =52）	13（25.00）	24（46.15）	3（5.77）	2（3.85）	1（1.92）	3（5.77）	0（0.00）	32（61.54）
χ^2/Fisher 值	0.212	1.368	0.074	0.044	–	0.074	–	0.003
P 值	0.645	0.242	0.786	0.834	1.000	0.786	0.441	0.956

组别	感染				
	呼吸道感染	真菌感染	皮肤溃疡	单纯疱疹病毒感染	合计
r–ATG 组（n =41）	7（17.07）	6（14.63）	1（2.44）	1（2.44）	14（34.15）
p–ALG 组（n =52）	10（19.23）	5（9.62）	2（3.85）	0（0.00）	17（32.69）
χ^2 值	0.071	0.177	0.044	–	0.022
P 值	0.789	0.640	0.834	0.441	0.883



附图　两组患儿的 Kaplan–Meier 生存曲线图

3　讨论

近年来，免疫介导造血抑制成为国内外对 AA 发病机制的普遍共识[10–12]。CsA 则可通过降血清雌二醇以及升高血清睾酮与网织红细胞计数水平促进免疫功能改善，对提升 AA 疗效有良好的辅助作用[13]。ATG/ALG 可以清除循环系统中的异常活化 T 细胞，研究表明其在调节细胞介导的白细胞 – 内皮细胞相互作用中也起了重要作用，在一定程度上刺激造血调节因子增

殖，促进造血干细胞增殖。故在 allo-HSCT 应用受限的情况下，CsA 与 ATG/ALG 的联合 IST 方案被大多数医疗机构作为 AA 的一线治疗手段。该方案通过多重机制重建造血，使 SAA 的治疗缓解率由上世纪 80 年代 <10% 的水平提升到了现阶段的 60% ~ 80%。但从 2011 年开始，大量文献指出不同种属来源的 ATG/ALG 存在一定疗效差异 [14-16]。临床医师对种属剂型的取舍决策或将对 SAA 的治疗预后产生直接影响。针对目前在国内使用的 r-ATG 和 p-ALG，已有研究分别证实了两者的有效性与安全性，然而关于 SAA 患儿的随机对照研究尚有不足。

本次随访结果显示 r-ATG 组与 p-ALG 组治疗 2 年后 OS 分别为 87.80% 与 84.62%，治疗 3 年后 OS 分别为 87.80% 与 78.85%；治疗 5 年后 OS 分别为 60.98% 与 53.85%，组间比较无差异，与近年国外其他剂型的 OS 相近 [17]。

作为异种球蛋白，ATG/ALG 所导致的过敏反应与血清病反应增高是其在临床应用中所必须承担的风险。此类过敏反应为Ⅰ型超敏反应，通常在第 2 次接触抗原后的数分钟内快速发生。国内相关报道显示，CsA 联合 ALG/ATG 治疗急性 AA 时的过敏反应发生率大概为 41%[18]。本研究 r-ATG 组与 p-ALG 组的过敏反应的发生率分别为 53.66% 与 32.69%，组间比较有差异，提示 p-ALG 较 r-ATG 在诱发过敏反应方面可能风险更低；同时从单项统计结果可以看出，过敏反应差异更集中体现在发热，具体原因尚不明确。另外，因严重过敏反应而导致治疗终止 p-ALG 组较 r-ATG 组发生率高。血清病是一类在接受异种血清制品后所出现的全身性Ⅲ型超敏反应，普遍发生于治疗后第 2 周内，临床表现主要包括有发热、皮疹及关节肿痛等。近 2 年国内相关文献显示 ALG/ATG 治疗 SAA 时的血清病发生率为 38.10% ~ 58.33%[19-21]。本研究中，r-ATG 组与 p-ALG 组的血清病发生率分别为 60.98% 与 61.54%，略高于文献报道，可能与辅助治疗中甲泼尼龙的给药剂量及时间有关。适当增加甲泼尼龙给药剂量或通过减慢滴速延长静滴时间或能在一定程度降低血清病发生率。

ALG/ATG 治疗对白细胞具有破坏作用，这将导致感染几率增加，其中又以呼吸道感染最为多见 [22]。在本研究过程中笔者确保患儿均在隔离的净化帐或清洁病房接受治疗，同时合理应用抗生素对抗感染，积极预防霉菌感染，结果显示 r-ATG 组与 p-ALG 组发生感染的比例分别为 34.15% 与 32.69%，低于其他学者的报道 [23]。此外，在既往关于 IST 治疗儿童 SAA 的报道中，个别患儿有出现复发或异常克隆转化的情况，严重者甚至可表现有向白血病转化的倾向。本研究开展后的 1 年密切随访未出现复发，也未发现异常克隆转化，这可能与随访时间不足或样本量偏小有关，目前仍在进一步随访观察当中。

综上所述，CsA 分别联合 r-ATG 或 p-ALG 治疗儿童 SAA 在缓解血液学反应方面效果相当。后续拟加大样本并进行两种制剂对细胞免疫相关生化指标的研究，以期在疗效信息与作用机制方面获得更为准确而丰富的认知。

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Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Predicting response to porcine antilymphocyte globulin plus cyclosporine A in children with acquired severe aplastic anemia

Li-Peng Liu[1], Xiao-Juan Chen[1], Wen-Yu Yang[1], Mei-Hui Yi[1], Kang Zhou[2], Min Ruan[1], Fang Liu[1], Xia Chen[1], Li-Xian Chang[1], Tian-Feng Liu[1], Li Zhang[1], Yao Zou[1], Yu-Mei Chen[1], Feng-Kui Zhang[2], Xiao-Fan Zhu[1] and Ye Guo[1]

BACKGROUND: In severe aplastic anemia (SAA), predictive markers of response to immunosuppressive therapy (IST) of porcine antilymphocyte globulin (pALG) have not been well defined. We investigated whether clinical and laboratory findings before treatment could predict response in a pediatric cohort.

METHODS: In this study, we included 70 newly diagnosed SAA children and treated them with pALG. The response rate was documented during follow-up. The log-rank test compared response rates between the potential predictive factors.

RESULTS: The response rate was 57.1% at 24 months follow-up. In log-rank test, mild disease severity was the most significant predictive marker of better response ($P < 0.001$); SAA patients with higher absolute reticulocyte count (ARC) and platelet level showed a higher response rate (both $P < 0.001$). Although insignificantly, elderly children and male sex show better response rate after treatment. The response rate worsened when the time interval before IST was more than 60 days.

CONCLUSION: Modified IST with pALG was suitable for SAA children, and favorable response correlates with mild disease severity was identified. ARC and platelet status also appeared to be a reproducible prognostic model for response rate. IST should be started as soon as possible, given that the response rate worsens as the interval between diagnosis and treatment increases.

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INTRODUCTION

Severe aplastic anemia (SAA) in children is a rare, life-threatening disorder characterized by pancytopenia and hypocellular bone marrow.[1] Over the past years, bone marrow transplantation (BMT) from a matched related donor has been the treatment of choice for children with SAA. For children without an available matched related donor, immunosuppressive therapy (IST) is indicated.[2]

IST is usually based on antithymocyte globulin/antilymphocyte globulin (ATG/ALG) polyclonal antibodies from animals' serum immunized by human thymocytes/lymphocytes.[3] The standard IST regimen involves horse ATG (hATG) and cyclosporine A (CSA).[4] In the 1980s and 1990s, previous studies in SAA conducted in the United States, Europe, and Japan utilized hATG, with hematologic responses observed in about two-thirds of cases.[5–7] According to previous experience, hATG yielded hematological improvement in 60–75% SAA patients after initial treatment,[8,9] and with 85% 5-year survival.[10] For salvaging patients who are refractory or relapse after initial therapy with hATG, treatment with rabbit ATG (rATG) results in a beneficial effect in about 60% of patients.[11,12] However, the use of rATG may not be quite as effective as first-line treatment involving hATG,[13] and hATG is currently unavailable in some countries including China.[3] Alternative ISTs have also been suggested to improve the efficacy of SAA treatment, and many different antibodies have emerged. One of these alternatives is porcine ALG (pALG), which is widely used in China, in part because it lowers the cost of therapy by 30–50% per cycle of treatment.[14] There have been many researchers reporting that the safety and efficiency of pALG are similar to hATG. The overall response rate (ORR) of IST with pALG plus CSA was 65–80% in adult.[15–17]

To date, only a few studies have considered the combination of pALG and CSA in separate severity of SAA children in terms of the hematopoietic response and failure-free survival (FFS) in 24 months. Moreover, we investigate whether clinical and laboratory findings before treatment could predict response in a pediatric cohort.

MATERIALS AND METHODS

Study population

A total of 70 children with SAA were enrolled from January 2015 to July 2018 at the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. The SAA diagnostic criteria were based on the International AA Study Group Criteria. SAA patients with malignant disease, myelodysplastic syndrome (MDS), myelofibrosis, paroxysmal nocturnal hemoglobinuria (PNH), or congenital AA-like Fanconi's anemia were excluded.

AA was considered severe if at least two of the following parameters were reached: platelet (PLT) count $<20 \times 10^9$/L,

[1]Division of Pediatric Blood Diseases Center, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 300020 Tianjin, China and [2]Division of of Anemia Therapeutic Center, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 300020 Tianjin, China
Correspondence: Xiao-Fan Zhu (xfzhu@ihcams.ac.cn)

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absolute neutrophil count (ANC) $<0.5 \times 10^9$/L, and a reticulocyte count $<15 \times 10^9$/L with hypocellular bone marrow.[18] Very severe AA (vSAA) was defined using the same three criteria, and ANC was $<0.2 \times 10^9$/L. Patients were considered to be in remission if: (1) there is no bleeding or anemia; (2) transfusion of blood products is not required; (3) white blood cell (WBC) counts $>3.5 \times 10^9$/L, PLT $> 100 \times 10^9$/L, ANC $\geq 1.0 \times 10^9$/L, the hemoglobin (Hb) level reaches remission standards, and (4) patients were in stable condition or had a substantial improvement after 24 months follow-up.

Immunosuppressive regimens

IST consisted of pALG (Wuhan Institute of Biological Products, China) intravenously 20 mg/kg/day followed by 1 mg/kg/day methylprednisolone on days 1–5 and then 1 mg/kg/day prednisolone for 15–21 days and tapered gradually to prevent serum sickness. CSA 3–5 mg/kg/day was used for at least 2 years.[3] The CSA dose was adjusted to achieve a whole-blood trough level of 100–200 ng/mL. Complete blood counts and biochemistry parameters were monitored accordingly.

Response criteria

Complete response (CR) was defined as independence from transfusion, associated with normal hemogram parameters. Partial response (PR) was defined as no longer meeting the criteria for SAA without normalization of blood counts and no transfusion dependence for PLT and/or red blood cells (RBCs). Continuous transfusion dependency and hematopoietic stem cell transplantation (HSCT) within 6 months of IST treatment before CR was classified as no response. Relapse was defined as a decrease in blood counts to values either requiring transfusions or needing reinstitution of IST or HSCT.[19,20] The responses to IST were evaluated at 40 days, 3, 6, 12, and 24 months after pALG administration. An overall response (OR) was defined as a CR or PR after initiation of IST. FFS was defined as the time from the first day of IST treatment until the relapse, disease- or treatment-related death, or the date of treatment failure, whichever came first.

Detection of PNH clone

We used a highly sensitive two-color flow cytometry method to detect PNH-type CD11b+CD55−CD59− granulocytes and glycophorin A+ CD55−CD59− RBCs. On the basis of data obtained in healthy population, a universal inter-laboratory threshold of ≥0.01% glycophosphatidyl inositol/FLAER-deficient cells with an apparent cluster-like distribution in granulocytes and RBCs was defined as a criterion for the presence of PNH-type cells.[21]

Statistical analysis

The FFS rate was analyzed using the Kaplan–Meier method. Differences in continuous variables were analyzed using the Mann–Whitney *U* test, and differences in frequencies were analyzed using Fisher's exact test. Log-rank test was used for the comparison between the potential predictive factors. All differences were considered significant at $P < 0.05$. Data were subjected to statistical analysis using SPSS 22.0 (SPSS Software Inc., Chicago, IL, USA) and GraphPad Prism 6.02 software (GraphPad Software Inc., La Jolla, CA, USA).

The study design and methods complied with the Declaration of Helsinki and was approved by the Ethics Committee and Institutional Review Board of Chinese Academy of Medical Sciences and Peking Union Medical College. Informed consent was obtained from all subjects.

RESULTS

We identified 70 patients (48 males, 22 females) who fulfilled the eligibility criteria during the study period. The median age of SAA children were 5 years (ranging from 1 to 14 years). Population characteristics and main baseline biological results are shown in Table 1.

Bone marrow biopsy was performed on all patients and all patients were transfusion dependent at the time of diagnosis. Overall survival (OS) rate during follow-up period was 100%. A total of 36 of the 70 patients (51.4%) achieved at least a CR or PR at 6 months after IST. There was a significantly higher proportion of response in the SAA group when compared with the vSAA group, 24 (60%) and 12 (40%), respectively ($P = 0.098$). There were two late responses separately, which occurred at the 12-month time point in both groups. Moreover, the ORR did not change in the 24-month time point in comparison to the 12-month time point. The difference in ORR between the SAA and vSAA group was insignificant at the end of the follow-up period ($P = 0.125$), which suggested that pALG is an effective treatment for all SAA patients (Table 2).

Treatment failure was observed in 14 patients in the SAA group and 16 patients in the vSAA group; at the end of follow-up, these patients underwent salvage therapies because of a lack of response to IST ($n = 16$) or relapse ($n = 14$). A total of 24 patients underwent HSCT as a salvage therapy. Among these patients, six received HSCT at the 3-month time point, 12 patients received HSCT at the 6-month time point, and six received HSCT at the 12-month time point.

To determine predictors of IST response, we compared differences in potential pre-treatment variables between IST responders and non-responders. The following were analyzed both for prevalence in categorical variables and differences in continuous variables: age at diagnosis, sex, time interval before treatment, PNH clone, absolute lymphocyte count (ALC), Hb level, absolute reticulocyte count (ARC), and PLT count. Table 3 indicated that PNH clone, severity of SAA and baseline ARC, and PLT level showed influence with IST response. We also performed multivariate logistic regression analysis to assess the simultaneous contributions of each of the variables in predicting response. In these analyses, higher ANC ($P = 0.0009$) and PLT count ($P = 0.0007$), shorter interval between diagnosis and therapy ($P = 0.019$), and PNH clone negative ($P = 0.009$) represented significant predictors of better response (Table 4).

Table 1. Population characteristics at diagnosis

Baseline characteristics	SAA ($n = 40$)	vSAA ($n = 30$)	*P* value
Median age at diagnosis (range)	6 (2–13)	5 (1–14)	0.495
Male (%)	28 (70.0)	20 (66.7)	0.766
Idiopathic (%)	36 (90)	28 (93.3)	0.622
Median duration before IST, days (range)	50 (26–175)	39 (26–120)	0.102
Median observation time, days (range)	474 (327–664)	446 (287–664)	0.528
PNH clone positive	8 (20)	2 (6.7)	0.115
Salvage HSCT	12 (30)	12 (40)	0.383
Blood count			
Absolute lymphocyte count ($\times 10^9$/L)	1.98 (0.71–4.80)	1.36 (0.26–3.03)	<0.001
Hemoglobin (g/dL)	64 (30–81)	65 (31–81)	0.302
Absolute neutrophil count ($\times 10^9$/L)	0.25 (0.01–0.65)	0.1 (0.01–0.2)	<0.001
Platelet ($\times 10^9$/L)	7 (1–13)	4 (1–18)	0.184
Absolute reticulocyte count ($\times 10^9$/L)	12.3 (2.6–48.7)	7.1 (1.3–18.7)	0.005

SAA severe aplastic anemia, *HSCT* hematopoietic stem cell transplantation, *PNH* paroxysmal nocturnal hemoglobinuria, *IST* immunosuppresive therapy

Table 2. Hematologic response rate after IST in different time point

	SAA ($n = 40$)	vSAA ($n = 30$)	*P* value
Response at 40 days after IST			
CR	0	0	
PR	30 (75%)	14 (46.7%)	
ORR (CR/PR)	30 (75%)	14 (46.7%)	0.015
Response at 3 months after IST			
CR	4 (10%)	4 (13.3%)	
PR	20 (50%)	4 (13.3%)	
ORR (CR/PR)	24 (60%)	8 (26.6%)	0.006
Response at 6 months after IST			
CR	8 (20%)	6 (20%)	
PR	16 (40%)	6 (20%)	
ORR (CR/PR)	24 (60%)	12 (40%)	0.098
Response at 12 months after IST			
CR	16 (40%)	12 (40%)	
PR	10 (25%)	2 (6.7%)	
ORR (CR/PR)	26 (65%)	14 (46.7%)	0.125
Response at 24 months after IST			
CR	16 (40%)	12 (40%)	
PR	10 (25%)	2 (6.7%)	
ORR (CR/PR)	26 (65%)	14 (46.7%)	0.125

SAA severe aplastic anemia, *IST* immunosuppressive therapy, *CR* complete response, *PR* partial response, *ORR* overall response rate

Table 3. The univariate analysis of predictive factors for response rate during follow-up

Factors	Subtype	Number (percentage)	Response rate	*P* value
Age	≤6 year old	40 (57.1)	50.0%	0.118
	>6 year old	30 (42.9)	67.7%	
Sex	Male	48 (68.6)	54.2%	0.357
	Female	22 (31.4)	36.3%	
Time interval before IST	≤60 day	42 (60.0)	61.9%	0.869
	>60 day	28 (40.0)	50.0%	
PNH clone	Positive	10 (14.3)	40.0%	0.005
	Negative	60 (85.7)	60.0%	
SAA subtypes	SAA	40 (57.1)	39.7%	<0.001
	vSAA	30 (42.9)	19.9%	
Baseline ALC	$\leq 1.5 \times 10^9$/L	30 (42.9)	28.3%	0.311
	$>1.5 \times 10^9$/L	40 (57.1)	38.3%	
Baseline ARC	$\leq 10 \times 10^9$/L	46 (65.7)	24.5%	<0.001
	$>10 \times 10^9$/L	24 (34.3)	51.2%	
Baseline Hb	≤60 g/L	22 (31.4)	34.4%	0.960
	>60 g/L	48 (68.6)	31.0%	
Baseline PLt	$\leq 10 \times 10^9$/L	62 (88.6)	26.9%	<0.001
	$>10 \times 10^9$/L	8 (11.4)	93.7%	

ARC absolute reticulocyte count, *PLt* platelet, *Hb* hemoglobin, *ALC* absolute lymphocyte count, *SAA* severe aplastic anemia

The incidence of adverse responses shown in Table 5 was basically not different between the SAA and vSAA group ($P > 0.05$). All were controllable, and recovered after symptomatic treatment.

Table 4. Multivariate analysis for IST response in SAA patients during follow-up

	χ^2 value	OR (95% CI)	*P* value
Sex	0.94	0.48 (0.11–2.09)	0.332
PNH clone	6.74	0.105 (0.02–0.58)	0.009
Time interval	5.53	0.14 (0.03–0.72)	0.019
Age	2.22	1.18 (0.95–1.48)	0.136
Hb	0.30	0.99 (0.94–1.04)	0.585
ANC	6.78	4.70 (1.47–15.08)	0.009
ARC	0.41	2.06 (0.22–18.80)	0.522
PLT	7.31	1.34 (1.08–1.66)	0.007

CI confidence interval, *OR* odds ratio, *IST* immunosuppressive therapy, *ARC* absolute reticulocyte count, *PLT* platelet, *Hb* hemoglobin, *ALC* absolute lymphocyte count, *SAA* severe aplastic anemia

Table 5. Adverse event after pALG treatment

Adverse response	SAA ($n = 40$)	vSAA ($n = 30$)	*P* value
Fever	24	12	0.098
Rash	30	16	0.059
Arthralgia	2	2	0.766
Edema	2	4	0.218
Infection	6	3	0.536
Bleeding	10	4	0.227
Hypertension	16	10	0.568
Hematuria	4	0	0.074
Hepatic and renal dysfunction	12	6	0.343
Mucosa lesion	6	2	0.278
Hypokalemia	12	10	0.766

SAA severe aplastic anemia, *vSAA* very severe aplastic anemia, *pALG* porcine antilymphocyte globulin

Figure 1 indicated that the FFS rate of the SAA group were significantly higher than the vSAA group ($P < 0.001$), which indicated a better prognosis of patients with higher ANC. None of the patients died during the 24 months follow-up.

DISCUSSION

IST using ATG/ALG and CSA has been considered as the initial treatment for SAA patients, since most patients lack a minimal residual disease or are not suitable candidates for HSCT due to the lack of access to the certain modality. In China, rATG was the only available agent before 2004, but some of the patients could not afford this expensive drug, which is not covered by any insurance. In recent years, an alternative is pALG, in part because it is less expensive than rATG. An adult trial comparing the pALG with rATG regimen showed that pALG had a significantly better OS rate than rATG and faster median time to achieve first effectiveness in SAA patients.[3] A recent study revealed a large difference in efficacy among patients of different age treated with IST: <20 years ($n = 31$, 100%), 20–40 years ($n = 51$, 92%), 40–60 years ($n = 51$, 71%), and >60 years ($n = 56$, 56%).[22] This finding suggests that the IST treatment results for pediatric and adult patients obtained should be analyzed separately, but the researchers work on this issue is rare.

This was the first and largest study to observe the outcomes following combination therapy with pALG and CSA in children

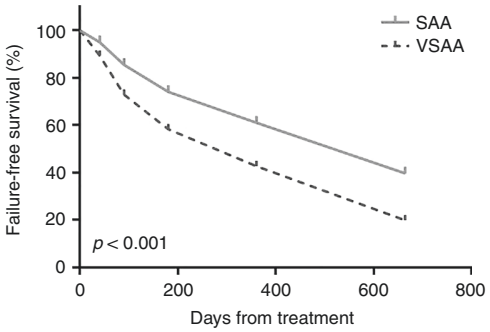

Fig. 1 The estimated failure-free survival (FFS) rate. During the 24 months follow-up, the FFS rates of the severe aplastic anemia (SAA) group was significantly higher than the very severe aplastic anemia (vSAA) group ($P < 0.001$)

with different severity of SAA, and compare differences in other potential pre-treatment variables between responders and non-responders. We identified a better FFS in the SAA group than in the vSAA group in the treatment of pALG during the 24 months follow-up, thus favorable response correlating well with higher ANC. We also performed univariate analysis to assess the contribution of each variable in predicting response. In these analyses, higher ARC and PLT level before IST, patients without PNH clone, and SAA group represented significant predictors of better response.

In this study, although response rate in the separate time point in the SAA group was basically higher than that in the vSAA group, there was no significant difference after 24 months in pALG treatment, which indicated the good effect in all SAA children. The adverse response after pALG treatment were moderate, so it is relatively safe. A previous study conducted by Cui et al.[23] reported the overall response to pALG regimen at 3, 6, and 12 months were 56, 64, and 62%, respectively, in SAA children, which were similar to our study.[23] However, they did not perform further analysis on the variables between responders and non-responders, as pre-treatment clinical and laboratory findings may exert an obvious influence on the response to IST. Thus, it was relatively incomplete.

Kulagin et al.[21] reported that the presence of PNH clones and ARC were identified as independent predictors for hematological response, which was also validated in our study. A number of previous studies in both adults and children with SAA found a high prevalence (up to 70%) of PNH clones at diagnosis and during IST, which has prompted recommendations for PNH clone screening among SAA patients in a number of guidelines.[24] Some studies indicated that the presence of small PNH clones in SAA patients is of potential importance, as it has been shown that these patients have a higher response to IST.[25] However, PNH positivity was lack of prognostic value according to the NIH group study.[26] In view of this, the value of PNH clone presence in the setting of pALG remains less conclusive. The current data confirmed a distinctly lower response rate in PNH-positive children after pALG regimen.

When it comes to the association between sex and response, we found that the response rate was better in the male group than in the female group, although the difference was not statistically significant, which was also observed in a retrospective study in another Asian cohort, where a young males group experienced faster recovery of bone marrow function following ATG.[27] Poor response in girls could be influenced by their incapacity to produce hemopoietic growth factor activity. Nissen et al.[28] previously reported that a high production of granulocyte colony is associated with a positive outcome after ALG treatment: hemopoietic reconstitution is invariably preceded by a bout of excess granulocyte colony. Accordingly, low granulocyte colony release, as observed in young girls, may herald poor response. Therefore, it appears that young females get a particular form of SAA characterized by low endogenous factor production and poor response to ALG.

Although insignificantly, response rates to IST were considerably low among SAA children with long-standing disease. Only 50% of patients treated more than 60 days after diagnosis responded, indicating that children may receive irreversible damage to hematopoietic progenitor cells, possibly due to immune attack through autoreactivated lymphocytes.[27] The present study suggests the importance of immediate treatment for patients and recommends offering IST as quick as possible in all SAA children who lack a matched sibling donor.

Regarding the association between disease severity and response, conflicting results have been reported. A European study reported superior response rates in children with vSAA compared to SAA,[29] but some studies including a recent report of a Korean cohort of adult patients have produced the opposite result.[30] The present findings were consistent with those published studies, with favorable responses correlating well with higher ANC rather than ALC. In children with AA, treatment ANC may mainly reflect the size of WBC populations, due to the severe neutropenia in this condition. These results suggest that better response to IST might possibly be ascribed to higher WBC count, that is, a relative increase in neutrophil. When ALC was applied to the analysis, no correlation between ALC and response rate was observed in this study.

In SAA children, pre-treatment ARC and PLT count may mainly reflect the status of hematopoiesis function. The present findings suggested that higher response to IST might possibly be ascribed to improved hematopoiesis function, that is, a higher baseline ARC and PLT count may indicate better residual marrow function and the presence of a sufficient number of stem cells to sustain blood cell production after IST.

OS rate during follow-up period was 100%, thus we also adopted FFS rates following IST in this study; it is much more important than survival alone for evaluating the long-term outcome.[31]

We also have limitations in this study. First, taking into consideration any variable in the study population, we cannot exclude the possibility that another variable may reflect better hematopoietic reserves in the study cohort. Second, although our data were statistically significant, the small sample size in the study suggests that larger SAA trials are needed to further confirm our results.

CONCLUSION

In conclusion, modified IST with pALG was suitable for all SAA children, and pre-treatment clinical and laboratory findings influence response to pALG and CSA. A better FFS in the SAA group than in the vSAA group in the treatment of pALG was identified during follow-up, thus favorable response correlates with higher neutrophil count or mild disease severity than lymphocyte count, and this blood count parameter might help in clinically assessing bone marrow function. ARC and PLT status also appeared to be a reproducible prognostic model for response rate in the setting of ALG and CSA for SAA children. It appears that young girls get a particular form of SAA characterized by low endogenous factor production and poor response to pALG. IST should be started as soon as possible after diagnosis of SAA, given that the response rate worsens as the interval between diagnosis and treatment increases.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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ORIGINAL ARTICLE

Modified immunosuppressive therapy with porcine antilymphocyte globulin plus delayed cyclosporine A in children with severe aplastic anemia

Qingya Cui[1] · Pingping Sha[1] · Haifei Chen[2] · Hongshi Shen[2] · Longmei Qin[2] · Zhengyang Li[2] · Tianqin Wu[2] · Zhaoyue Wang[1]

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Abstract Immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine (CsA) is the standard treatment for children with severe aplastic anemia (SAA) with no human leukocyte antigen-matched siblings. Due to the unavailability of horse ATG in China, porcine antilymphocyte globulin (p-ALG), which is less expensive and more effective than rabbit ATG, is widely used. We sought to evaluate the efficacy and safety profile of modified IST with p-ALG plus delayed CsA at day 21 in 50 SAA children. Eighteen SAA patients who progressed from nonsevere aplastic anemia (NSAA) were classified as SAA-II; the other 32 patients were classified as SAA-I. Overall response (OR) rates at 3, 6 and 12 months were 56, 64 and 62%, respectively. The 10-year overall survival (OS) rate and disease-free survival (DFS) rate were 80 and 56%. The OR, OS and DFS rates in the SAA-I group were clearly better than those in the SAA-II group. Death rate from infection within 30 days was 4%. Modified IST with p-ALG plus delayed CsA is a reliable and well-tolerated treatment for children with SAA, and reduces early death due to infection. Modified IST is more suitable for children with SAA-I.

Keywords Severe aplastic anemia · Porcine antilymphocyte globulin · Modified immunosuppressive therapy · Infection

Introduction

Acquired aplastic anemia (AA) is an immune-mediated destruction of bone marrow stem and progenitor cells characterized by bone marrow aplasia and peripheral blood pancytopenia [1]. Due to a less frequent and severe graft-versus-host disease (GVHD) and better overall survival (OS), the outcome of hematopoietic stem cell transplantation (HSCT) in AA children is superior to that in adults [2, 3]. However, the majority of children with severe aplastic anemia (SAA) lack a human leukocyte antigen (HLA)-matched sibling donors, the standard immunosuppressive therapy (IST) regimen with antithymocyte globulin (ATG) plus cyclosporine (CsA) remain the first treatment option for those patients with SAA [4].

Since the 1980s, porcine antilymphocyte globulin (p-ALG) has been used to treat patients with SAA in China, there have been many laboratory researches and clinical observations showing that the safety and efficiency of p-ALG are similar as other ATGs. The overall response rate of IST regimen with p-ALG plus cyclosporine was 65–80% in adult [5–11]. Although p-ALG is also widely used for pediatric patients with SAA in China, systematic investigation of this preparation in the treatment of pediatric patients with SAA was seldom reported. In addition, CsA therapy usually begins at the time as ATG intravenous infusion. The simultaneous combination of these two immunosuppressive agents would increase the risk of severe infections [6, 7, 11]. We hypothesize that the modified IST with p-ALG plus delayed CsA would reduce death rate especially for

The authors Qingya Cui and Pingping Sha contributed to the work equally.

✉ Tianqin Wu
wtq100@vip.163.com

✉ Zhaoyue Wang
zwang11@sina.com

[1] Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Key Lab of Thrombosis and Hemostasis of Ministry of Health, 188 Shizi Street, Suzhou 215006, China

[2] The Department of Hematology, PLA 100th Hospital, 53 Wuqueqiao Street, Suzhou 215006, China

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severe infections. In this study, we investigated the therapeutic effect, safety profile, and the long-term outcome of the modified IST in pediatric patients with SAA.

Patients and methods

Ethics statement

This study was approved by the Ethics Committee of the 100th Hospital of People's Liberation Army. Patient information was anonymized and their parents consented to allow the use of their child medical records for research.

Subjects

50 SAA children under the age of 18 years who had no HLA-matched sibling were enrolled from January 2003 to January 2011 at Department of hematology, the 100th Hospital of People's Liberation Army, Suzhou, Jiangsu province, China. All diagnoses were reviewed by at least three senior hematologists independently. At least one time of ilial crest punch biopsy and two times of marrow aspiration were included in the diagnosis of AA. Marrow cytogenetic studies were performed for all patients, and the patients with clonal cytogenetic abnormalities were excluded in this study. Fanconi anemia, paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS) and other constitutive bone marrow failure syndromes were excluded. Hepatitis and Epstein-Barr virus (EBV)-related pancytopenia were also excluded before IST. Inherited AA with characteristic physical findings, such as short stature, abnormal thumbs, hypopigmented and café au lait spots, was excluded in physical examination [4].

Definitions

SAA was defined by the presence of a hypocellular bone marrow (cellularity less than 25%) and at least two of the following three criteria from peripheral blood counts: platelet counts $\leq 20 \times 10^9$/L, neutrophil count $\leq 0.5 \times 10^9$/L, and corrected reticulocyte count $\leq 1\%$. The disease was classified as very severe aplastic anemia (VSAA) if the neutrophil count was $< 0.2 \times 10^9$/L. Nonsevere aplastic anemia (NSAA) was defined as condition in which patients did not fulfill the criteria for SAA or VSAA. The patients with SAA who developed from NSAA were considered as SAA-II. Complete response (CR) was defined as neutrophil count $> 1.5 \times 10^9$/L, platelet count $> 100 \times 10^9$/L, and hemoglobin (Hb) >100 g/L. Partial response (PR) was defined by transfusion independence and an increase in hematological parameters from baseline values with at least Hb >70 g/L, a neutrophil count $> 0.5 \times 10^9$/L and platelet count $> 30 \times 10^9$/L. Patients were considered in relapse if blood counts decreased to values either requiring transfusions or needing reinstitution of IST. Clonal evolution was defined when a new clonal disorder appeared on cytogenetic analysis or bone marrow analysis showed the characteristic morphologic changes [18].

Immunosuppressive regimens

Immunosuppressive therapy consisted of p-ALG (20–30 mg/kg/day for 5 days), CsA (5 mg/kg/day since day 21 to maintain for 24 h per day for 10 consecutive days, and then switched to 3–8 mg/kg/day divided into two doses for oral treatment), and metacortandracin (2 mg/kg/day for 5 days) with subsequent halving of the dose every week until discontinuation on day 28. If child had uncontrolled infection after administration of p-ALG, administration of CsA was delayed further. The CsA concentration was maintained at 200–300 μg/mL at least 6 months and the dosage was gradually reduced when the maximum effect was achieved. The total course of treatment with CsA was 1–2 years. If child had p-ALG-related serologic reaction (including p-ALG-related fever, rash, arthralgia, hemolysis), metacortandracin dose was increased. Granulocyte colony-stimulating factor (G-CSF) was administered for children at 5 mg/kg/day subcutaneously and the dose was adjusted with neutrophil counts. The patients were carefully monitored for any infection and were given appropriate antibiotics in the event of infection. The patients were given transfusions of blood components to maintain safe blood counts, in compliance with the policies of the local medical center.

Statistical methods

The Fisher Exact test or the Chi-Square test and the Student *t* test were used to calculate the significance of differences between SAA-I and SAA-II groups. The overall survival and disease-free survival (DFS) were evaluated by the Kaplan–Meier method. DFS was calculated from the date of IST to the first day of either recurrence or clonal transformation or death. *p* value less than 0.05 was considered to be statistically significant. All statistical analyses were performed by SPSS 13.0 software.

Results

Patients' characteristics

A total of 50 SAA patients received p-ALG plus CsA as IST protocol. The male/female ratio was 1.08. Median age at diagnosis was 14 years (range 5–17 years). Median time from diagnosis to IST was 16 days (range 14–32 days) in

Table 1 Patients' characteristics

	SAA-I (32)[a]	SAA-II (18)[a]	*p* value[b]
Average age (year)	13.63 ± 3.47	14.22 ± 2.44	0.216
Average duration (months)	1.36 ± 1.14	54.67 ± 37.32	0.000
Peripheral blood cell count before treatment			
Absolute neutrophil count ($\times 10^9$/L)	0.37 ± 0.26	0.34 ± 0.26	0.866
Platelet count ($\times 10^9$/L)	13.19 ± 8.20	10.00 ± 4.97	0.121
Hemoglobin (g/L)	60.88 ± 21.79	52.89 ± 22.25	0.426

[a]Plus-minus values are mean ± SD

[b]*p* value was calculated by the *t* test

Table 2 50 SAA patients response to modified IST

	SAA (50)	SAA-I (32)	SAA-II (18)	*p* value[b]
Overall response[a]				
3 months (*n*/%)	26/(56%)	18/(56.25%)	8/(44.44%)	0.052
6 months (*n*/%)	32/(64%)	24/(75%)	8/(44.44%)	0.031
12 months (*n*/%)	31/(62%)	24/(75%)	7/(38.89%)	0.012
Relapse	5/(10%)	3/(9.38%)	2/(11.11%)	0.599
Clonal evolution	3/(6%)	1/(3.16%)	2/(11.11%)	0.291
Death				
Total death (*n*/%)	10/(20%)	4/(12.5%)	6/(33.3%)	0.083
Early death (*n*/%)	4/(8%)	2/(6.25%)	2/(11.1%)	0.456
Later death (*n*/%)	6/(12%)	2/(6.25%)	4/(22.2%)	0.114

[a]Overall response = complete response + partial response

[b]Compare between SAA-I and SAA-II, *p* value was calculated by Chi-square test

Table 3 Serious complications after p-ALG treatment and prognosis

Serious complications after p-ALG	Case (*n*)		Prognosis
	Total	Death	Recovery
Pulmonary infection	5	2	3
Bloodstream infection	3	0	3
Acute appendicitis	1	0	1
Perianal infection	1	0	1
Wide epifolliculitis	1	0	1
Cerebral hemorrhage	2	2	0
Hematuria	2	0	2
Hepatotoxicity	3	0	3
Autoimmune hemolytic anemia	3	0	3
Capillary leak syndrome	1	0	1

SAA-I group and 12 months (range 12–120 months) in SAA-II group. The median neutrophil count was 0.38×10^9/L (range 0.08–1.1×10^9/L); median reticulocyte count was 10.1×10^9/L (range 0.6–21×10^9/L) and median platelet count was 11×10^9/L (range 2–42×10^9/L). All the patients were idiopathic SAA and 64% (32/50) of them were classified as SAA-I group. All patients received the first IST treatment. The patients' characteristics are shown in Table 1.

Response to therapy

The overall response rates at 3 months, 6 months and 12 months were 56% (SAA-I 56.25%, SAA-II 44.4%, $p = 0.052$), 64% (SAA-I 75%, SAA-II 44.4%, $p = 0.031$) and 62% (SAA-I 75%, SAA-II 38.89%, $p = 0.012$), respectively (Table 2).

Safety evaluation

Common side effects after p-ALG treatment were hypersensitivity reactions and serum sickness (20 cases), characterized by fever (12 cases), mild rash (3 cases), or joint pain (5 cases), and these side effects could be controlled by non-steroidal agent and glucocorticoid. There were 10 patients with severe infections during period of IST treatment, including 5 cases of pneumonia, 3 cases of bloodstream infection, 1 case of acute appendicitis and 1 case of wide folliculitis. Two children died of severe pulmonary fungal infection in the early and two children died of cerebral hemorrhage for severe platelet transfusion refractoriness. There was one case of capillary leak syndrome induced by p-ALG, which was rare side reaction and controlled by glucocorticoid, plasma transfusion and artificial colloid. Three patients appeared autoimmune hemolytic anemia during the use of p-ALG, and were controlled by glucocorticoid treatment and transfusions of washed red blood cells (Table 3).

Relapse and clonal evolutions

The cumulative incidence of relapse in 10 years was about 10%. Three (6%) of 50 children evolved into MDS.

Survival

The median follow-up time for surviving patients was 59.6 months (range 11–176.8 months). The 10-years OS rate was 80% (SAA-I 87.5%, SAA-II 66.7%, $p = 0.037$) (Fig. 1).

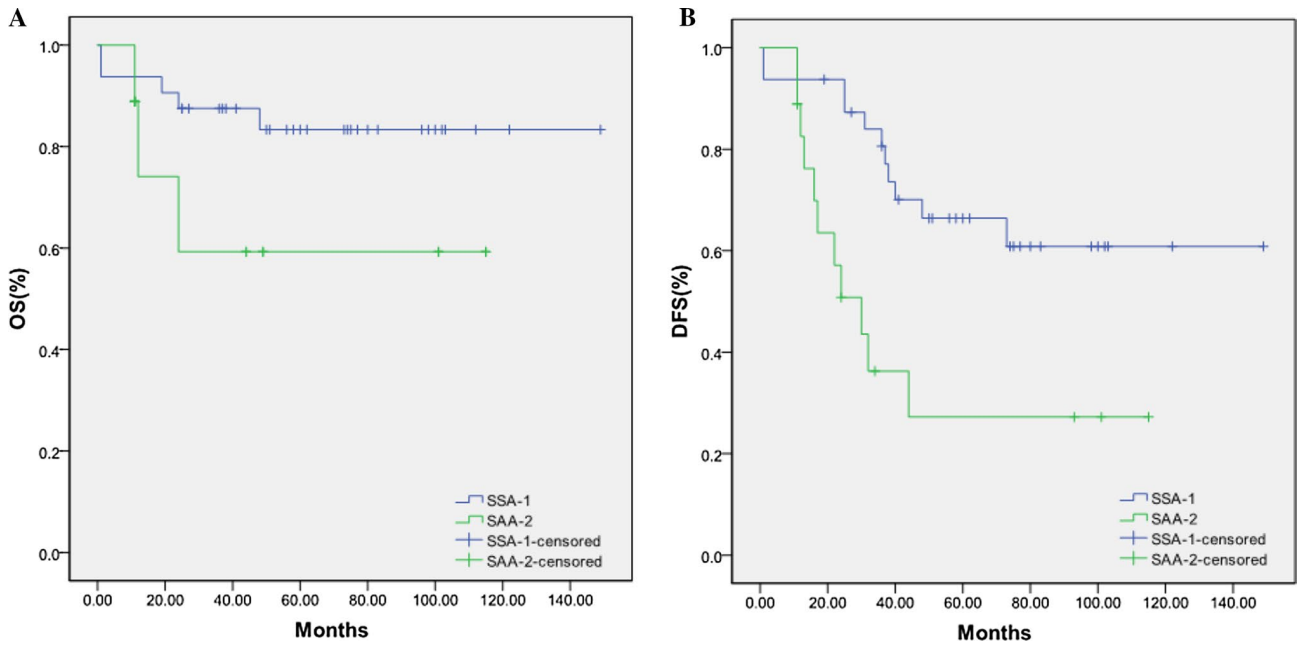

Fig. 1 Comparison of the 10-years overall survival (OS) and disease-free survival (DFS) rate between the SAA-I group ($n = 32$) and the SAA-II ($n = 18$) (A and B). The 10-years OS and DFS rate in the SAA-Igroup were significantly higher compared to the SAA-II group ($p < 0.05$)

The 10-years DFS rate was 56% (SAA-I 65.6%, SAA-II 38.9%, $p = 0.003$) (Fig. 1).

Discussion

The incidence of AA in China is higher than that in Europe and North America, but the economic situation of patients in China is worse than that of patients in developed countries [6]. In pediatric patients with SAA who have an HLA-matched sibling donor, HSCT is often the preferred treatment for the superiority of transplantation over IST in children [12]. Due to the limitations of HLA-matched sibling donor and high cost of ATG, IST with p-ALG plus CsA is a major treatment strategy in China. In the previous studies, response rates and long-term survival of pediatric patients with AA was 58–80 and 80–90% when treated with the combination of ATG + CsA [13–17]. In our retrospective study, the children response rates to combination of p-ALG and CsA were 56, 64 and 62% in 3, 6 and 12 months, respectively. Response rate in the 3, 6 and 12 months in the SAA-I was 56.25, 75 and 75%, obviously higher than that in SAA-II group. The long-term survival of pediatric patients was approximately 87.5% in SAA-I group, significantly higher than 66.7% in SAA-II group. Therefore, our modified IST is more suitable for children with SAA-I and the efficacy is not lower than that in other clinical researches.

In most clinical studies, the early death rate of IST was mostly above 10% due to severe infection. In our study, there were two deaths (4%) due to severe pulmonary fungal infection and another two patients (4%) died of cerebral hemorrhage for severe transfusion refractoriness in SAA-I group. Hypersensitivity reactions and serum sickness after p-ALG treatment, including fever, mild rash, joint pain and autoimmune hemolytic anemia, were moderate and could be controlled by non-steroidal agent and glucocorticoid. There was a rare secondary reaction of capillary leak syndrome resulting from p-ALG and that was controlled by glucocorticoid, plasma, and artificial colloid. Therefore, modified IST is relatively safe.

The cumulative incidence of relapse at 10 years was about 10%, lower than other published data in western countries [18–20], and quite similar with adult SAA patients treated with combination of p-ALG and CsA in China [7, 9].

Although clonal evolution may occur in approximately 10–15% of patients [21], incidence rate was 0–7.8% in Chinese SAA patients treated with combination of p-ALG and CsA [5, 7, 8]. Only three (6%) of 50 children evolved into MDS.

In our study, the average duration of 18 SAA-II patients developing from NSAA was 54.67 ± 37.32 months. Over time, hematopoietic stem cell reserve was ongoing draining and replaced by more fat in the SAA-II patients, that may be contributed to the lower response rate and OS rate in patients of SAA-II.

In summary, our retrospective results show great safety and efficiency of p-ALG plus with delay the usage of CsA as the first-line IST in the treatment of acquired SAA in

children. The modified IST is more suitable for SAA-I patients and seems to be of the great advantage of relatively low early death of infection.

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Compliance with ethical standards

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抗淋巴细胞球蛋白和抗胸腺细胞球蛋白治疗儿童重型再生障碍性贫血的不良反应比较

马秀慧　赵文理　卢俊　王易　胡绍燕　何海龙

【摘要】　目的　比较抗胸腺细胞球蛋白（ATG）及抗淋巴细胞球蛋白（ALG）在治疗儿童重型再生障碍性贫血（SAA）中的不良反应。**方法**　回顾性分析 132 例诊断为 SAA 并接受 ALG/ATG 为主的联合免疫抑制治疗患儿的临床资料，对不良反应发生率进行比较，组间有效率比较应用 χ^2检验。**结果**　59 例使用 ALG，发生过敏反应 19 例（32.2%），其中出现发热 6 例（10.17%），因严重过敏反应而停止治疗者 4 例（6.78%）；发生血清病反应 36 例（61.01%），其中发热 14 例（23.73%）；19 例（32.20%）出现不同程度的感染，以呼吸道感染最常见（16.95%）。73 例使用 ATG，过敏反应 37 例（50.68%），22 例（30.14%）出现发热，2 例（2.74%）因严重过敏反应而停用；血清病反应 45 例（61.64%），其中发热 21 例（28.77%）；出现感染 23 例（31.50%），同样呼吸道感染多见（21.91%）。经统计分析，ATG 较 ALG 发生过敏反应更高，其中以发热反应有显著统计学差异（$P<0.05$）；而在出现严重过敏反应导致停药，ALG 组较 ATG 更多见，但无显著统计学差异；血清病反应及感染两者未表现出显著差异（$P>0.05$）。**结论**　过敏反应、血清病及感染为治疗中常见的不良反应。ATG 较 ALG 更容易引起 SAA 患儿发生过敏反应，其中以发热反应发生率更高，而血清病反应及感染发生率无差异。

【关键词】　贫血，再生障碍性；　儿童；　抗淋巴细胞球蛋白/抗胸腺细胞球蛋白；　副反应

再生障碍性贫血（aplastic anemia，AA）是由多种病因引起的以全血细胞减少为主要临床表现的造血功能衰竭综合征，是儿童难治性血液病之一[1]。重型再生障碍性贫血（severe aplastic anemia，SAA）发病机制中免疫因素占重要地位，联合免疫抑制治疗是对无条件进行造血干细胞移植患者的主要治疗方案，其经典药物为抗淋巴细胞球蛋白（ALG）/抗胸腺细胞球蛋白（ATG）联合环孢霉素A（Cyclosporin A，CsA），治疗有效率为 60%～80%[2-4]。国外已有报道马抗人胸腺细胞球蛋白（H-ATG）无论在成人还是儿童SAA疗效均优于目前常用的兔抗人胸腺细胞球蛋白（R-ATG）[5]；但国内制剂常用的仍为猪抗人淋巴细胞球蛋白（P-ALG）与兔抗人胸腺细胞球蛋白（R-ATG）。其两者在副作用、疗效比较相对报道少见。本文对ALG及ATG治疗的 132 例SAA患儿资料进行了总结，对其不良反应进行了分析。

一、资料和方法

1. 一般资料：2009 年 2 月至 2013 年 6 月，132 例患儿诊断为 SAA 并接受 ALG/ATG 治疗，诊断标准参照文献[6]，其中 59 例使用 ALG，55 例接受了完整的 ALG 疗程，有 4 例未能完成疗程。73 例使用 ATG，71 例完成了 ATG 疗程，1 例因出现过敏性休克而改为使用 ALG，1 例因第 4 天出现脉氧饱和度低而停用末次 ATG。

2. 治疗方案：（1）药品来源；P-ALG（武汉生物制品研究所，250 mg/支）；R-ATG（法国赛达药厂，25 mg/瓶 。（2）联合免疫治疗：行 ALG 皮试后，ALG 以静脉滴注 20 mg•kg^{-1}•d^{-1}×5 d；ATG 以静脉滴注 3.75 mg•kg^{-1}•d^{-1}×5 d。ATG 或 ALG 治疗后用 CsA 5 mg•kg^{-1}•d^{-1}，po，q12 h，并根据血药浓度调整剂量，使之维持在 150～250 ng/dl。（3）辅助及支持治疗：所有患者均入住层流净化帐，并辅以雄激素（40～80 mg/d，po，qd）治疗；酌情行成分输血及应用粒细胞集落刺激因子等造血生长因子度过危险期。（4）不良反应防治：全部病例应用 ATG 或 ALG 过程中均联用甲泼尼龙。治疗前建立 2 条静脉通道，一条静滴 ATG 或 ALG，另一条静滴甲泼尼龙 2 mg•kg^{-1}•d^{-1}，其中 1 mg/kg 在 ATG 或 ALG 使用 30 min 静滴，余 1 mg/kg 同时维持，用药结束后再予甲泼尼龙 1 mg/kg 口服或静滴，四周内逐渐减停。

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作者单位：215003　江苏省，苏州大学附属儿童医院血液科
通讯作者：何海龙，Email: blood.he@yahoo.com.cn

3. 观察指标：对患儿治疗期间及其后一个月内的不良反应进行观察，其中包括类过敏反应、使用后的血清病反应及感染情况。发热出现的时间和伴随症状具有判断意义。凡在应用 ATG 或 ALG 疗程结束至第 2～3 周出现发热等症状者，如伴有充血性皮疹、关节痛等，而无明显感染征象，应首先考虑为 ATG 或 ALG 所致血清病，给予泼尼松龙静滴，并酌情行血培养，病毒、支原体抗体测定，X 线胸片、头颅 CT 或腹部超声以明确有无感染。

4. 统计学分析：应用 SPSS 16.0 统计学软件进行分析，组间有效率比较应用 χ^2检验，$P<0.05$ 认为有统计学意义。

二、结果

1. 类过敏反应（表 1）：（1）ALG 组：59 例患儿中 19 例出现类过敏反应，发生率为 32.2%。主要表现为发热 6 例（10.17%）、皮疹和皮肤瘙痒 15 例（25.42%）、呛咳、咽部不适 2 例（3.39%），关节疼痛肿胀 1 例（1.69%）。发生时间为 ALG 使用期间 1～5 d，绝大多数为用药第 1 天。4 例（6.78%）因严重过敏反应而停止治疗，包括急性溶血 3 例，1 例使用第 3 天出现肌肉疼痛，下肢肿胀，随后出现器官功能损害，诊断为过敏反应导致的渗漏综合征，虽然经积极治疗，但出现血压下降等 DIC 表现，伴心力衰竭，经抢救无效而死亡。（2）ATG 组：73 例患儿中 37 例出现类过敏反应，发生率为 50.68%。主要表现为发热 22 例（30.14%）、皮疹和皮肤瘙痒 22 例（30.14%）、呛咳 2 例（2.74%），头痛、血压升高 3 例（4.11%）。发生时间为 ATG 使用期间 1～5 d，绝大对数仍为使用第 1 天。其中 1 例因使用当天出现过敏性休克而改用 ALG，1 例因使用第 4 天出现脉氧饱和度低而停用末次 ATG。

经统计分析，ATG 较 ALG 更容易出现类过敏反应，其中以发热反应差异具有统计学意义（$P<0.05$）；而在皮疹、呛咳、关节肿痛及头痛等方面均无统计学差异；在出现严重过敏反应导致停药，ALG 组较 ATG 多见，但无显著统计学差异。

2. 血清病反应（表 2）：（1）ALG 组：59 例患儿中 36 例出现不同程度的血清病反应，发生率 61.01%。血清病的主要表现发热、皮疹、关节肿痛、肺部渗出、溶血、腹腔积液。其中发热 14 例（23.73%），皮疹伴瘙痒 27 例（45.76%），关节肿痛 3 例（5.08%），肺部渗出 2 例（3.39%）、腹腔及盆腔积液 2 例（3.39%），血压升高、头痛 3 例（5.08%），急性溶血 0 例。发生时间为 ALG 治疗结束后 2～19 d，第 2～7 天最集中。（2）ATG 组：73 例患儿中 45 例出现不同程度的血清病反应，发生率 61.64%。其中发热 21 例（28.77%），皮疹伴瘙痒 26 例（35.62%），关节肿痛 1 例（1.37%），胸腔积液 3 例（4.11%）、腹腔积液 1 例（1.37%），黏膜损害 3 例（4.11%）、头痛 3 例（4.11%）。发生时间为 ATG 使用结束后 3～13 d，第 4～8 天最集中。

统计分析认为，两者在发生发热、皮疹、关节肿痛及肺部渗出等血清病反应中均未表现出显著差异。

表 1 ALG 与 ATG 使用中类过敏反应的比较[例，(%)]

组别	例数	发热	皮疹、瘙痒	呛咳	关节肿痛	头痛	终止	合计
ALG	59	6(10.17)	15(25.42)	2(3.39)	1(1.69)	0	4(6.78)	19(32.2)
ATG	73	22(30.14)	22(30.14)	2(2.74)	0	3(4.11)	2(2.74)	37(50.68)
χ^2值		7.784	0.359	0.047	1.247	2.356	1.227	4.563
P 值		0.005	0.549	0.828	0.264	0.125	0.268	0.033

表 2 ALG 与 ATG 使用后血清病的比较[例，(%)]

组别	例数	发热	皮疹、瘙痒	关节肿痛	肺部渗出	腹腔、盆腔积液	头痛	黏膜损害	合计
ALG	59	14(23.73)	27(45.76)	3(5.08)	2(3.39)	2(3.39)	3(5.08)	0	36(61.01)
ATG	73	21(28.77)	26(35.62)	1(1.37)	3(4.11)	1(1.37)	3(4.11)	3(4.11)	45(61.64)
χ^2值		0.425	1.398	1.532	0.053	0.599	0.072	2.481	0.005
P 值		0.514	0.237	0.216	0.817	0.439	0.789	0.115	0.941

表 3 ALG 与 ATG 使用后感染表现的比较[例，(%)]

组别	例数	呼吸道感染	真菌感染	单纯疱疹病毒感	败血症	合计
ALG	59	10(16.95)	4(6.77)	2(3.39)	2(3.39)	19(32.20)
ATG	73	7316(21.91)	5(6.85)	2(2.74)	0	23(31.50)
χ^2值		0.51	0.00	0.04	2.51	0.00
P 值		0.47	0.98	0.82	0.11	0.93

3. 感染（表 3）：（1）ALG 组：所有病例治疗前均排除感染迹象，在使用药物后 1 个月内对 59 例患儿进行观察，出现不同程度的感染 19 例（32.20%）。其中以呼吸道感染最常见，包括不同程度肺炎 7 例（11.8%）（除外真菌感染）、其他呼吸道感染 3 例（5.1%）（支气管肺炎 2 例、上呼吸道感染 1 例）；口腔及肺部真菌感染 4 例（6.77%）；皮肤单纯疱疹病毒感染 2 例（3.39%）；败血症 2 例（3.39%）。予抗感染及对症支持治疗后患儿均得以控制。（2）ATG 组：同样对 73 例患儿使用药物后一月内进行观察，出现感染 23 例（31.50%），同样呼吸道感染多见，其中肺炎（除外真菌）9 例（12.3%），其余呼吸道感染 7 例（9.6%）；口腔及肺部真菌感染 5 例（6.85%）；皮肤单纯疱疹病毒感染 2 例（2.74%）。

统计分析显示，两者在使用药物后出现感染均以呼吸道感染多见，在口腔及肺部真菌、单纯疱疹病毒感染及败血症等方面均未表现出统计学差异。

三、讨论

目前在我国因异基因同胞骨髓移植来源困难和费用昂贵等问题限制其广泛应用的情况下，联合免疫抑制治疗已成为 AA 患者的一线治疗手段[7-8]，主要药物包括 ATG/ALG 及 CsA 在内的许多免疫抑制剂。AA 的免疫介导发病机制已经成为国内外的普遍共识，采用 ATG/ALG 为主的联合免疫抑制治疗的远期疗效也已经得到充分肯定[9-11]。其通过抑制 T 淋巴细胞，减低 T 淋巴细胞产生的造血负调控因子，解除此类因子对造血细胞抑制和（或）破坏，重建造血，使 SAA 的治疗缓解率由 20 世纪 80 年代的 10%以下提高到 60%～80%[2-4]。美国血液学会多中心临床研究 AA-92 报道 ATG 为主的联合免疫抑制治疗 18 周岁以下 AA，6 个月总有效率为 71%[12]，均证实了联合免疫抑制治疗的有效性。目前，联合免疫抑制治疗重点关注各剂型不良反应及疗效有何差别。本文对 ATG/ALG 治疗儿童 SAA 临床不良反应进行了比较。

ATG/ALG 为异种球蛋白，类过敏反应和血清病反应较高的发生率，成为临床使用过程中担心的风险所在。过敏反应为Ⅰ型超敏反应，发生速度快，一般发生在第 2 次接触抗原后数分钟。国内有报道 ALG/ATG 联合 CsA 治疗急性 AA 所致类过敏反应为 41%[13]；而 ALG 为主的免疫抑制剂治疗 AA 致过敏反应约 38.9%至 44%[14-15]；以 ATG 为主的联合免疫抑制治疗 AA 致过敏反应约 34.4%[16]；本文资料显示，132 例患儿中，56 例发生类过敏反应，总发生率为 42.4%；59 例使用 ALG，19 例出现过敏反应，发生率为 32.2%；73 例使用 ATG，37 例出现过敏反应，发生率 50.68%，与文献报道相仿。本组病例中，ATG 较 ALG 更易引起过敏反应，其中以发热反应有显著差异；而在出现严重过敏反应终止治疗中 ALG 组较 ATG 更多见，但无显著统计学差异。

血清病是指因异种血清制品而导致的全身的Ⅲ型超敏反应性疾病，Ⅲ型超敏反应可溶性抗原与相应抗体结合，形成可溶性免疫复合物，沉积于毛细血管基底膜，激活补体系统引起以充血水肿、中性粒细胞浸润、组织坏死为主要特征的病理性免疫应答，又称免疫复合物型或血管炎型超敏反应。临床主要表现为发热、皮疹、关节痛等。国内报道使用抗胸腺球蛋白/抗淋巴细胞球蛋白治疗SAA发生血清病反应为52.0%～68.0%[2,4,15,17]。本文资料显示，132例患儿中，81例发生血清病反应，总发生率为61.36%；59例使用ALG，36例出现血清病反应，发生率为61.01%；73例使用ATG，45例出现不同程度的血清病反应，发生率61.64%，与文献报道相仿，且两者无显著差异。

出现上述不良反应，或通过减慢滴速使静滴时间延长至 12 h 以上，或给予甲泼尼龙加量，症状可很快内消失。本组资料也显示相同结果，仅有少数患者出现严重不良反应，说明免疫抑制治疗近期过敏反应及血清病反应发生率高，但通过给予及时处理，可以耐受。

ATG/ALG 可以破坏白细胞可致各种严重感染。国内有相关报道[18]，感染发生率为 50%左右，其中主要为呼吸道感染。但因各地区治疗环境不同，给予的防御措施存在差异等原因，感染发生率不尽相同。本组病例所有患者均入住层流净化帐，但仍有 30%左右赶热闹发生。从发生的类型分析，呼吸道感染多见，所以注重环境保护对患儿具有重要意义。有真菌感染及带状疱疹出现，可在使用 ALG/ATG 早期给予一定真菌预防及人血丙种球蛋白输注，可以减少此类感染发生。严重感染抑制骨髓造血恢复，使病情加重，病程延长，形成恶性循环。故在治疗 AA 过程中，应高度重视防治感染，尤其潜伏病毒和真菌感染。

本文比较了以 ATG/ALG 为主的联合免疫抑制

治疗治疗 SAA 患者不良反应，主要包括类过敏反应、血清病反应及感染。ATG 较 ALG 更易引起 SAA 患儿出现过敏反应，其中 ATG 较 ALG 更容易引起发热反应；两者未表现出血清病反应及感染差异。及时应用甲泼尼龙等处理，可显著减少不良反应发生。以 ATG/ALG 为主的联合免疫抑制治疗治疗儿童 SAA 的疗效情况及造血恢复速度等，值得我们研究，我们将在以后的工作中做进一步阐述。

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儿童和成人输血依赖性非重型再生障碍性贫血的免疫抑制法疗效分析

项静静, 林圣云, 沈建平, 叶宝东, 沈一平, 胡致平, 郑智茵, 周郁鸿, 王珺

【摘要】目的　探讨免疫抑制法(IST)治疗儿童和成人的输血依赖非重型再生障碍性贫血(TD-NSAA)的疗效及安全性。方法　回顾性分析149例(其中儿童组33例,成人组116例)TD-NSAA患者的临床资料。结果　儿童组和成人组以ATG/ALG为主,有效率、脱离输血例数、脱离输血时间、无事件生存期(EFS)均明显优于CSA组(均$P<0.05$),且儿童组总脱离输血时间较成人快($P<0.05$),儿童组ATG/ALG为主的EFS明显高于成人组(均$P<0.05$),成人组和儿童组ATG/ALG或CSA治疗后总生存期(OS)差异均无统计学意义(均$P>0.05$)。并发症:ATG/ALG为主的方案发生血清病高于CSA组($P<0.05$),但进展为SAA的风险少于CSA组($P<0.05$)。结论　TD-NSAA治疗方案以ATG/ALG为主的免疫抑制治疗方案优于CSA治疗方案,且儿童疗效优于成人。

【关键词】　输血依赖;再生障碍性贫血;免疫抑制治疗;儿童;成人
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再生障碍性贫血(AA)是由造血干/祖细胞(HSPC)自身免疫性破坏所引起的骨髓造血功能衰竭症(BMF)。根据骨髓和血象分为重型再生障碍(SAA/VSAA)及非重型再生障碍(NSAA),部分NSAA患者长期依赖成分输血称之为输血依赖的NSAA(TD-NSAA)[1],对于SAA/VSAA推荐异基因造血干细胞移植(allo-HSCT)及抗人胸腺/淋巴细胞球蛋白(ATG/ALG)为主的免疫抑制法(IST)一线治疗方案[2]。Jeong等[3]报道IST治疗TD-NSAA的有效率为68.1%。传统研究多以治疗方案进行分组,临床上多认为儿童IST治疗疗效明显优于成年人,但缺乏相应的文献支持。本研究回顾性分析儿童和成人TD-NSAA患者两种治疗方案的疗效,现将结果报道如下。

1　资料与方法

1.1　一般资料　选取浙江中医药大学附属第一医院2006年1—12月收治的TD-NSAA 149例,均符合NSAA诊断标准[4-5],同时有严重贫血,活动受限或极易活动性出血,需每月1次及以上成分(红细胞或血小板)输血者。将患者分为儿童组和成人组,两组除了年龄不同外,其他基线特征差异均无统计学意义(均$P>0.05$)。见表1。

1.2　治疗方案　(1)ATG/ALG:22例(儿童5例)采用兔ATG 3～5 mg·kg^{-1}·d^{-1}×5 d,47例(儿童11例)采用国产猪ALG 20～30 mg·kg^{-1}·d^{-1}×5 d,同时予以糖皮质激素预防过敏。ATG治疗后使用CsA 3～5 mg·kg^{-1}·d^{-1}序贯治疗。(2)环孢霉素A(CSA):80例(儿童17例)采用CSA 3～5 mg·kg^{-1}·d^{-1}。支持治疗:辅以成分输血、重组人粒细胞集落刺激因子(G-CSF)、重组人促红素注射液(EPO)、注射用重组人白介素-11(IL-11)、重组人血小板生成素注射液(TPO)、雄激素及抗感染等。

1.3　观察指标　采用Camitta[6]的再生障碍疗效评价标准,比较两组有效率、脱离输血时间、总生存期(OS)及无事件生存期(EFS)。

1.4　统计方法　采用SPSS 19.0统计软件进行分析,计量资料以中位数(P25,P75)表示,两两比较采用Mann-Whitney U检验;计数资料比较采用χ^2检验或Fisher确切概率法;生存曲线采用Kaplan-Meier及Log-Rank法。$P<0.05$为差异有统计学意义。

2　结果

2.1　儿童组和成人组疗效比较　两组内ATG/ALG治疗后有效率、脱离输血时间、脱离输血的例数均明显优于CSA(均$P<0.05$),且儿童组开始脱离输血时间早于成人组($Z=2.773$,$P<0.05$);两组组间总有效率、总脱离输血例数、总存活率及组内存活率差异均无统计学意义(均$P>0.05$)。见表2。

2.2　生存曲线

2.2.1　儿童组及成人组组内生存曲线比较　儿童及成人组ATG/ALG与CSA治疗后中位OS分别为[97.5(56,132)个月 *vs* 45(13,120)个月,$P=0.148$]和[46(6,132)个月 *vs* 46(12,132)个月,$P=0.095$],差异无统计学意义。儿童组及成人组ATG/ALG为主组的中位EFS均优于CSA[80(0,128)个月 *vs* 0(0,81)个月,$P=0.005$]和[47(0,132)个月 *vs* 0(0,96)个月,$P=0.016$],差异有统计学意义。

2.2.2　ATG/ALG及CSA组间比较　儿童组和成人组的中位OS差异均无统计学意义(ATG/ALG组间:$P=0.339$;CSA组间:$P=0.862$)。儿童ATG/ALG为主组EFS高于成人组($P=0.041$),CSA组EFS差异无统计学意义($P=0.847$)。

2.3　并发症　儿童组及成人组组内及组间发生爆发性肝炎、脓毒性休克、重要脏器出血、转化为MDS/AML、PNH差异

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作者单位:310006杭州,浙江中医药大学附属第一医院
通信作者:林圣云,Email:lsyww2012@163.com

表 1 TD-NSAA 患者的基线特征比较

基线特征	儿童组(n=33)		成人组(n=116)	
	ATG/ALG(n=16)	CSA(n=17)	ATG/ALG(n=53)	CSA(n=63)
中位年龄(岁)	14(10,16.75)	16(8,18)	30(24,37)	35(26,45)
男/女	7/9	7/10	27/26	32/31
诊断至治疗时间(月)	10(7.5,11.8)	10(7.5,12.5)	10.5(7,12.5)	11(7.5,13)
红细胞输注依赖(例)	2	2	7	8
血小板输注依赖(例)	2	3	8	9
红细胞和血小板均输注依赖(例)	12	12	38	46
IST 方案[例数(%)]				
r-ATG	5(31.25)	—	17(32.08)	—
P-ALG	11(68.75)	—	36(67.92)	—
诊断时外周血常规				
ANC(10^9/L)	1.05(0.525,1.5)	1(0.75,1.15)	1(0.7,1.4)	0.9(0.7,1.33)
HGB(g/L)	61(50.5,74.75)	56(51.5,57.5)	58(51.5,69)	56(51,62)
PLT(10^9/L)	14.5(8.25,19.5)	13(7,18)	14(8,22.5)	11(7,16)
RET(10^9/L)	28.85(23.25,3175)	31(21.1,36)	31(27,35)	31(24,37)
肝功能异常(例)	1	1	5	5

注：r-ATG：兔抗人胸腺淋巴细胞免疫球蛋白；p-ALG：猪抗人胸腺淋巴细胞免疫球蛋白；ANC：中性粒细胞；HGB：血红蛋白；PLT：血小板；RET：网织红细胞

均无统计学意义(均 $P>0.05$)；无论儿童还是成人 ATG/ALG 治疗后血清病发生的风险增加，进展为 SAA 风险少于 CSA，且差异均有统计学意义(均 $P<0.05$)，但儿童组与成人组组间差异无统计学意义(均 $P>0.05$)。见表 3。

表 2 儿童组和成人组疗效比较

治疗方法	儿童组(n=33)		成人组(n=116)	
	ATG/ALG(n=16)	CSA(n=17)	ATG/ALG(n=53)	CSA(n=63)
有效率(%)	81.25	41.17*	77.36	41.27 a
脱离输血例数	13	7*	40	26 a
脱离输血时间(d)	52(45,62)	101(99,116) b	68.5(61.25,80.75)	120(98,128.5) b
存活率(%)	87.5	76.47	86.79	73.02

注：与 ATG/ALG 相比，a$\chi^2\geq$12.55，b$Z\geq$3.614，*为使用 Fisher 确切概率法，$P<0.05$

表 3 儿童组和成人组并发症比较

组别	治疗方法	爆发性肝炎	脓毒性休克	重要脏器出血	血清病	PNH	转化为 MDS/AML	进展为 SAA
儿童组	ATG/ALG(16 例)	0	0	0	5	1	0	2
	CSA(17 例)	0	0	0	0*	0	0	9*
成人组	ATG/ALG(53 例)	1	3	0	11	5	2	7
	CSA(63 例)	1	8	3	0a	1	2	23a

注：与 ATG/ALG 相比，a$\chi^2\geq$8.15，*为使用 Fisher 确切概率法，$P<0.05$

3 讨论

TD-NSAA[1]为依赖成分输血＞1 次/月的 NSAA，目前国内外还没有统一治疗方案，2016 年英国指南上[6]提出可首选 ATG/ALG 为主的 IST 治疗；中国 2017 年专家共识提出 TD-NSAA 先用 CSA 治疗，观察 6 个月无效再选择移植或 ATG/ALG 为主的方案[7]。TD-NSAA 患者长期输血，易并发感染和向 SAA 或 VSAA 疾病进展，一旦转化为 SAA 或 VSAA，疗效极差。TD-NSAA 患者治疗时机和方案的选择是临床研究者一直探索的课题。

本研究显示，TD-NSAA 无论是成人还是儿童以 ATG/ALG 为主 IST 的有效率、脱离输血时间、脱离输血例数、EFS 均优于 CSA 组(均 $P<0.05$)，且儿童组总脱离输血时间明显早于成人组(P=0.006)。虽然儿童组和成人组组内及组间治疗后 OS 差异均无统计学意义(均 $P>0.05$)，但是儿童组 ATG/ALG 为主治疗后 EFS 优于成人组(P=0.041)，说明 ATG/ALG 为主的治疗方案，患者生存质量优于 CSA 组。两组免疫抑制剂方案并发症差异无统计学意义(均 $P>0.05$)，但 ATG/ALG 为主治疗后向 SAA 转化的概率减少。

综上所述，对于 TD-NSAA，以 ATG/ALG 为主的 IST 优于 CSA，且儿童组疗效优于成人，虽然差异无统计学意义，可能与儿童组病例数较少有关。笔者将继续扩大病例，开展前瞻性研究，为 TD-NSAA 的治疗提供更加有力的证据。

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初治青少年急性早幼粒细胞白血病使用成人方案的疗效分析

张碧波,陆滢

【摘要】目的　探究初治青少年急性早幼粒细胞白血病使用成人方案的疗效。方法　回顾性分析33例初治青少年急性早幼粒细胞白血病使用成人方案的缓解率、总体生存率和非复发生存率,并与同时期成人初治急性早幼粒细胞白血病的疗效进行比较。结果　青少年组和成人组缓解率分别为96.97%和95.95%,青少年组和成人组患者K-M生存曲线估测的5年总体生存时间均未达到,两组5年总体生存率、5年非复发生存率差异均无统计学意义(均$P>0.05$)。结论　初治青少年急性早幼粒细胞白血病患者使用目前的成人方案,疗效并不逊色成人。

【关键词】　青少年;急性早幼粒细胞白血病;疗效
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急性早幼粒细胞白血病(APL)是一种有着特异基因PML-RARα融合基因或染色体核型改变t(15;17)的特殊类型急性白血病,临床表现凶险,起病及治疗过程中容易发生出血和感染而引起死亡。由于诱导治疗中维甲酸(ATRA)及亚砷酸(As_2O_3)的使用,近年来APL的预后明显改善,但是对于部分高危患者,疾病缓解后还是容易复发。青少年APL患者高白细胞比例高,长期治疗出现脏器损害的可能性大。笔者给予33例青少年APL患者ATRA联合As_2O_3诱导治疗,以及包含蒽环类药物的巩固维持治疗,并与同时期成人患者进行比较,以探究成人方案对青少年APL的疗效,报道如下。

1　资料与方法

1.1　一般资料　选取宁波市鄞州人民医院2005年1月至2015年11月住院的初诊青少年APL患者33例,其中男23例,女10例;年龄12～18岁,平均(16.06±1.71)岁。同时期成年患者74例,其中男29例,女45例;年龄19～81岁,平均(38.54±16.33)岁。均符合相关诊断标准[1],患者入院前未接受任何治疗,肝肾功能正常,无重要脏器如心肺的并发症。

青少年组中位白细胞(WBC)2.3×10^9/L,中位血小板(PLT)20×10^9/L;基因亚型L型17人,S型16人;根据文献[2]对患者进行危险分层:低危(WBC≤10×10^9/L,PLT＞40×10^9/L)5例,中危(WBC≤10×10^9/L,PLT≤40×10^9/L)20例,高危(WBC＞10×10^9/L)8例。成人组APL中位WBC 3.05×10^9/L,中位PLT 24.5×10^9/L,基因亚型L型45人,S型29人;低危19例,中危32例,高危23例。两组基础资料差异无统计学意义($P>0.05$)。

1.2　治疗方法　(1)诱导缓解治疗:患者均接受口服ATRA联合As_2O_3诱导缓解治疗。ATRA 25 mg·m^{-2}·d^{-1},分3次口服,直至完全缓解;As_2O_3 0.16 mg·kg^{-1}·d^{-1},静脉滴注,直至完全缓解。诱导期间给予高三尖酯碱(HHT)1～3 mg/d,开始使用时间为白细胞计数超过5×10^9/L,在白细胞计数小于20×10^9/L且逐渐下降时停用,一般用药3～10 d。巩固维持治疗:诱导缓解后中低危患者给予去甲氧柔红霉素(IDA)8 mg·m^{-2}·$d^{-1}\times3$ d,高危患者同时加用阿糖胞苷(Ara-c)100 mg·m^{-2}·$d^{-1}\times7$ d,第2个月均口服ATRA 2周,第3个月均给予As_2O_3 0.16 mg·kg^{-1}·$d^{-1}\times28$ d,每轮为1个疗程,连续3年左右,第1、2、3年各疗程间隔分别为1、2、3个月,总计8个疗程。(2)中枢神经系统白血病的防治:在诱导缓解后开始接受预防性鞘内注射。地塞米松5 mg,阿糖胞苷30 mg/m^2,甲氨蝶呤10～15 mg,中低危者至少6次,高危者至少8次。(3)支持治疗:血小板＜50×10^9/L者输注新鲜血小板,有凝血功能障碍者给予新鲜冰冻血浆等,监测出凝血功能直至凝血功能正常;怀疑感染者立即送检相关分泌物培养和血液培养,同时给予足量广谱抗生素控制感染,取得药敏结果后调整抗感染治疗方案;As_2O_3治疗时监测肝肾功能、电解质、心电图;出现分化综合征则暂停ATRA、As_2O_3,并给予糖皮质激素,病情缓解后继续治疗。

1.3　观察指标　随访至2017年10月。复发率为治疗过程中复发患者例数与随访总例数的比值,总体生存时间(OS)为自确诊之日至死亡或者随访截止时间。非复发生存时间(RFS)为从取得完全缓解时间至随访结束、复发或者其他疾病引起死亡的时间,疗效及复发的判断依据文献[1]。患者在入院时和缓解后监测骨髓染色体和PML/RARα融合基因,获得完全缓解后每3个月复查1次骨髓PML/RARα融合基因至治疗结束后2年。

1.4　统计方法　应用SPSS 19.0统计软件进行分析,计量资料以均数±标准差表

作者单位:315040 宁波,宁波市鄞州人民医院
通信作者:陆滢,Email:luying741107@qq.com

专题笔谈·血细胞减少症与血小板减少症

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再生障碍性贫血分型及免疫抑制治疗选择

何广胜

摘要：中性粒细胞绝对值为0的暴发型再生障碍性贫血（FAA）使用免疫抑制治疗（IST）效果欠佳，一线选择异基因造血干细胞移植（allo-HSCT）可能更好；输血依赖型非严重型再生障碍性贫血（TD-NSAA）易进展为严重型AA（SAA），IST较单用环孢菌素（CsA）治疗则更易见效；老年SAA需根据年龄、合并症等分层给予IST联合艾曲泊帕等促血小板生成素激动剂治疗。

关键词：再生障碍性贫血；分型；免疫抑制治疗

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Classification of aplastic anemia and choice of immunosuppressive therapy. *HE Guang-sheng. Department of Hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing 210029, China*
E-mail: heguangsheng1972@sina.com

Abstract: The efficacy of immunosuppressive treatment (IST) in fulminant aplastic anemia (SAA) with absolute neutrophil value of 0 is not good, and allogeneic hematopoietic stem cell transplantation (allo HSCT) might improve the outcomes of patients; Transfusion dependent non severe aplastic anemia (TD-NSAA) progress to severe AA (SAA) forwardly, and IST could works better than CsA alone for TD-NSAA; Elderly SAA should be treated with IST combined with hrombopoietin receptor agonist according to age and complications.

Keywords: aplastic anemia; classification; immunosuppressive therapy

再生障碍性贫血（aplastic anemia，AA）于19世纪首次报道，目前已经将AA由一个全血细胞减少的综合征界定为是T淋巴细胞介导的以骨髓造血系统为靶器官的自身免疫性疾病。

1　暴发型再生障碍性贫血

依据外周血细胞减少的情况，AA分为3型：严重型AA（severe AA，SAA）、极重型AA（very severe AA，vSAA）和非严重型AA（non-severe AA，NSAA）。SAA患者的血常规具备下列3项中的2项或2项以上：中性粒细胞绝对值（absolute neutrophil count，ANC）<0.5×10^9/L；血小板（platelet，PLT）计数<20×10^9/L；网织红细胞（reticulocyte，Ret）计数<20×10^9/L。达到SAA标准，且ANC<0.2×10^9/L则为vSAA；达不到SAA标准的为NSAA[1]。

SAA和vSAA患者若无合适供体或不适合行异基因造血干细胞移植（allogeneic hematopoietic stem cell transplantation，allo-HSCT），一线治疗方案就是抗人胸腺细胞免疫球蛋白（anti-thymocyte globulin，ATG）和环孢菌素（closporin A，CsA）组成的免疫抑制治疗（immunosuppressive treatment，IST）。起初研究发现ANC对AA预后影响显著，NSAA预后优于SAA[2]，SAA优于vSAA[3]。但随着抗真菌和支持治疗进展，AA患者IST预后显著改善[4]，后续大宗临床数据登记分析显示ANC在成人SAA和儿童AA患者的IST中已不能指导预后[5-6]，甚至有报道儿童vSAA的IST效果优于SAA[7]。

那么，是不是ANC在IST中已无预后作用？日本儿童AA工作组数据显示，在ATG治疗前后2周的ANC为0患者，IST的6个月有效率差于vSAA和SAA（40.0%比63.6%比63.7%，*P*=0.027），但5年无事件生存率（failure-free survival，EFS）和总生存率（overall survival，OS）差异不显著（EFS：48.2%比

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作者单位：南京医科大学第一附属医院 江苏省人民医院血液科，江苏 南京 210029

通信作者：何广胜，电子信箱：heguangsheng1972@sina.com

65.1% 比 68.1%， P=0.206；OS：88.5% 比 95.8% 比 96.8%，P=0.281）[8]，据此，提出了ANC为0的暴发型AA（fulminant AA，FAA）的概念。但ATG对中性粒细胞有消耗作用，分型是为了判断预后和指导治疗，ATG之后根据ANC下降的再分型，在预后判断上指导作用不强。IST疗效与年龄成负相关，在成人SAA中ANC为0患者是什么情况，FAA的标准该如何界定？

中国东部贫血协作组对登记的成人SAA资料分析，并严格定义了FAA：（1）在IST或allo-HSCT之前，外周血白细胞涂片分类连续2次ANC为0；（2）对粒细胞集落刺激因子（granulocyte colony stimulating factor，G-CSF）连续应用3 d，无反应。研究发现FAA较vSAA、SAA不仅IST后6个月疗效最差（42.1%、60.9% 和 72.7%，P=0.028），OS也最差（63.2%、91.3% 和 95.5%，P=0.001）[9-10]。既然FAA的IST预后差，是不是即使年龄较大的同胞供体，或者有合适替代供体的allo-HSCT可以选择一线治疗？我们同期对照了19例allo-HSCT和28例IST治疗FAA发现，allo-HSCT后完全缓解率（CR）73.7%，部分缓解率（PR）10.5%，显著优于IST的10.7%、20.4%（总有效率84.2%比31.1%，P<0.001），2年OS也是allo-HSCT组为佳（84.2%比46.4%，P=0.021）（数据待发表）。因此，对于FAA治疗建议若可能一线选择allo-HSCT。

2 输血依赖型非严重型再生障碍性贫血

NSAA患者在3个血细胞严重减少指标中1项达到SAA标准：ANC<0.5×10^9/L，或PLT< 20×10^9/L，或Ret <20×10^9/L，并且血红蛋白（HGB）≤60 g/L，每月输注红细胞≥2 U的患者或有明显出血症状需输注血小板防治出血者，称为输血依赖型非严重型再生障碍性贫血（transfusion dependent non severe aplastic anemia，TD-NSAA）[11]。有学者提出Ret计数既往是基于人工涂片计算的结果，目前均已采用仪器分析，精确且稳定，建议仪器分析Ret计数以<60×10^9/L为红系造血严重减少标准[5]。

住院AA患者中TD-NSAA占近2/3，比例较高，值得关注。CsA治疗有效率仅19.3%，病程迁延，铁超负荷、肝肾功能损害、糖尿病或糖耐量异常及感染并发症常见，说明CsA治疗TD-NSAA疗效不佳，预后差。但ATG联合CsA有效率达47.3%，多因素分析提示ATG较单用CsA治疗更易见效[12]。Marsh等[13]关于成人NSAA研究显示，CsA有效率为46%、进展率29.5%，也表明CsA效果不够好；同期进行的ATG联合CsA研究有效率达74%、进展率14.8%，提示对于NSAA，尤其是TD-NSAA，一线采用ATG联合CsA是较佳选择[1]。allo-HSCT在治疗TD-NSAA中地位如何，值得积累数据分析。46.0%的TD-NSAA在中位时间24个月（3~216个月）进展为SAA，中性粒细胞绝对值<0.5×10^9/L、严重感染和铁超负荷是SAA进展的危险因素（P=0.022、P=0.025、P=0.001）[12]，对这类患者尤其应考虑一线选择ATG或allo-HSCT，可能会改善预后。

3 老年严重型再生障碍性贫血

AA发病率在60岁以上人群形成一个高峰[1,14]，尽管年龄不是老年SAA（≥60岁）采用ATG联合CsA的限制因素，但年龄确实与SAA的IST临床疗效的负相关[14]。欧亚955例AA患者回顾性分析显示老年SAA的5年长期生存率仅38%[15]，瑞典同期数据亦为38.1%[16]，提示老年SAA治疗确实是一个挑战。

老年SAA的ATG治疗相关毒副反应更大、风险更高。起初老年SAA单用CsA或减量使用ATG，发现效果不佳[14]；在能耐受标准剂量IST 6个月有效率达59%，与年轻患者类似[17]。艾曲泊帕是促血小板生成素受体激动剂（thrombopoietin receptor agonist，TPO-RA），与IST联合使用能增加SAA有效率和起效速度[18]，随后即开展了老年SAA中IST与艾曲泊帕联合使用。研究发现，IST单用或联合艾曲泊帕治疗3个月、6个月、9个月的有效率分别为9% 比47.8%（P=0.03），27.3%比72.7%（P=0.025），30%比79.2%（P=0.002）。高龄（≥70岁）和高查尔森合并症指数（≥5分）患者的疗效不佳（60%比40%，P=0.011；73.3%比26.7%，P=0.033）[19]。多因素分析发现：使用艾曲泊帕（P=0.012）、中性粒细胞计数（P=0.035）与IST疗效正相关，而年龄（P=0.004）、查尔森合并症指数（P=0.022）与疗效负相关；高查尔森合并症指数（P=0.041）和低中性粒细胞计数（P=0.035）对患者生存不利[19]。

因此，对于老年SAA有必要根据患者年龄、合并症，乃至体能情况、认知功能状态等进行综合评估后分层，分别给予足量或减量IST联合艾曲泊帕治疗，或支持治疗等。

总之，目前研究显示对于不同类型和疾病状态的AA分型和治疗选择应更加精细化，FAA一线推荐方案是allo-HSCT，TD-NSAA是ATG联合CsA，老年SAA需要进一步综合分层，予以足量或减量IST。艾曲泊帕等TPO-RA与不同IST方案组合将能极大改善AA治疗面貌。

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REVIEW ARTICLE

Current insights into the treatments of severe aplastic anemia in China

Lei Ye[1] · Fengkui Zhang[1] · Seiji Kojima[2]

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Abstract
Recently, several studies have been conducted to generate considerable evidence regarding unique treatments for severe aplastic anemia (SAA) in China. Haploidentical donor hematopoietic stem cell transplantation (HID-HSCT) showed an overall survival rate (80.3–86.1%) comparable to those with immunosuppressive therapy (IST) and matched related donor (MRD)- and matched unrelated donor (MUD)-HSCT. Failure-free survival of HID-HSCT was also comparable (76.4–85.0%) to those of MRD- and MUD-HSCT and better than IST in patients < 40 years. Although these results are promising, HID-HSCT should be regarded as a salvage therapy when young patients fail to respond to IST. Porcine anti-human lymphocyte immunoglobulin (pALG) showed similar or superior overall response at 6 months compared to rabbit anti-human thymocyte immunoglobulin (rATG) (64.0–79.4% in the pALG-group vs.48.1–64.7% in the rATG-group) as a first-line IST. Promising hematological response (28.4–33.3%) was observed in patients with refractory AA following infusion of the mesenchymal stromal cells (MSCs) derived from the bone marrow of allogeneic donors. pALG can replace rATG as an immunosuppressive drug and MSCs infusion can be used as a second-line treatment for refractory SAA. We believe that this review contributes to refine the global practices for SAA treatment.

Keywords Severe aplastic anemia · Haploidentical hematopoietic stem cell transplantation · Mesenchymal stromal cells · Porcine anti-human lymphocyte immunoglobulin

Introduction

Severe aplastic anemia (SAA) is a rare, life-threatening disease characterized by pancytopenia and hypocellular bone marrow. The annual incidence of AA appears to be 2–3 times higher in Asia than in Europe and North America, where the annual incidence rates are approximately 2 patients per million [1]. A nationwide cooperative study revealed that the annual incidence was 7.4 patients per million in China [2]. The large population size and higher incidence of AA in China translate into a very large number of new SAA patients being admitted to the tertiary hospitals. For example, more than 200 new patients have been admitted to the institute of Hematology and Blood Diseases in Tianjin, every year.

The choice of treatment for AA is determined as per the disease severity, patient age, donor availability, and access to the therapies. The current guidelines recommend bone marrow transplantation from a human leukocyte antigen (HLA)—matched related donor (MRD) as the treatment of choice, with a majority of recent studies showing an overall survival (OS) rates > 80% [3]. Immunosuppressive therapy (IST) using antithymocyte globulin (ATG) and cyclosporine A (CyA) has been indicated for young patients who do not have a MRD and those aged > 40 years. The Chinese government had employed the one-child policy for population control; therefore, it is difficult to find an HLA-matched donor within the patient's family. In China, allogeneic hematopoietic transplantation from a haploidentical related donor (HID-HSCT) has developed and has become the most common approach for allogeneic HSCT in SAA patients. Most patients received T-replete myeloablative HID-HSCT with granulocyte colony-stimulating factor(G-CSF)-primed bone marrow and peripheral blood hematopoietic stem cell, in contrast to the commonly used practices in other countries.

✉ Seiji Kojima
kojimas@med.nagoya-u.ac.jp

[1] Anemia Therapeutic Center, Institute of Hematology and Blood Diseases Hospital, CAMS & PUMC, Tianjin, China

[2] Department of Pediatrics, Nagoya University Graduate School of Medicine, 65, Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan

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The transplant indications for AA also differ from those in the Western countries. Unique AA treatments have been developed by Chinese doctors not only in the field of HSCT, but also in the field of IST, such as the use of porcine anti-human lymphocyte immunoglobulin (pALG) and mesenchymal stroma cells (MSCs). This review aimed to introduce these unique treatments and establish an algorithm for SAA treatment based on the available clinical evidence in China. We believe that this review contributes to refine the global practices for SAA treatment.

Haploidentical hematopoietic stem cell transplantation

In China, the number of SAA patients who underwent HID-HSCT has increased rapidly from 18 patients in 2008 to 268 patients in 2015, constituting 47% of the total allogeneic HSCTs for SAA [4]. In Europe, 60 HID-HSCTs were performed in 2017 for SAA (i.e., 10% of all allogeneic HSCTs for SAA) [5]. HID remains a minor donor source in countries other than China. The currently available data suggests a very favorable outcome of HID-HSCT in China. A study from 52 pediatric patients who underwent HID-HSCT as a first-line or salvage treatment showed that the 3-year OS rate and failure-free survival (FFS) rate were 84.5% and 82.7%, respectively. The cumulative incidence (CI) of grade II–IV acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD) were 39.2% and 34.2%, respectively. Transplantation-related mortality (TRM) at day + 100 and year + 1 was 13.5 ± 4.7% and 15.5 ± 5.0%, respectively. Causes of death included regimen-related toxicity(RRT) (one case), GVHD (three cases), graft failure (two cases), cerebral fungal infection (one case) and cerebral hemorrhage (one case) [6]. Another study on 51 adult patients showed 3-year OS and FFS rates of 83.5% each. The CI of grade II–IV aGVHD and cGVHD was 20.0% and 25.9%, respectively.The CI of TRM was 13.73 ± 0.24%. The cause of TRM was related with severe GVHD, infection and cardiotoxicity in one, four and two cases, respectively [7]. Conditioning regimens for both studies were busulfan (Bu), cyclophosphamide (Cy), and ATG. The stem cell source was a combination of recombinant human granulocyte (G-CSF)-mobilized bone marrow (BM) and peripheral blood. The GVHD prophylaxis comprised CyA, mycophenolate mofetil (MMF), and short-term methotrexate (MTX). The characteristics of the studies are summarized in Table 1. These results suggest that both, newly diagnosed and refractory SAA patients (both, adult and pediatric patients) would benefit from HID-HSCT. Further, Bu, Cy, and ATG was a safe and effective conditioning regimen for SAA patients.

Xu et al. compared the treatment outcomes between upfront HID-HSCT ($n = 76$) and upfront IST ($n = 37$) in adult patients. The estimated 8-year OS rate was comparable between the IST and HID HSCT groups (75.6% vs. 83.7%); however, the FFS rate was significantly lower in the IST group (38.5% vs. 83.7%, $p = 0.001$). In terms of blood recovery, 83.1% of the patients in the HSCT group showed complete recovery (CR) compared to only 38.2% in the IST group [8]. A superior FFS rate was also observed for HID-HSCT than for IST in pediatric patients (35.9% vs. 80.0%, $p = 0.043$) [9]. However, in patients > 40 years, no significant difference was observed in either the OS rate or the FFS rate between the two groups [8]. Moreover, the FFS rate was significantly lower in the salvage group than in the upfront group (41.7% vs. 80.0%, $p = 0.046$) [9]. FFS is much more important than OS for evaluating the long-term outcomes. A lack of response, relapse, and clonal evolution are problems associated with IST, while graft failure, aGVHD, cGVHD and secondary malignancies limit the success of HSCT. In the researches above [8, 9], FFS after HSCT was defined as death, primary and secondary graft failure and relapse, not including aGVHD and cGVHD. Even they defined the GVHD-free, failure-free survival (GFFS), was 72.4 ± 5.7% in the report of Xu et al. [8], prescribe a limit to grade 3–4 aGVHD, extensive cGVHD, and treatment failures. So far, as Bu and Cy, which may affect fertility, was the common conditioning regimen for HID-HSCT, there is no even one study compared the effect of haploid transplantation and immunosuppressive therapy on fertility function, which is very important especially in the young patient's whole life. Further studies are needed to confirm these findings. Moreover, other reports of IST treated SAA and VSAA in China, FFS of 5 and 10 years were 67% [10, 11] and 50–60% [12, 13], respectively. These data indicate the need to rethink the treatment options for SAA.

Another study compared upfront MRD-HSCT ($n = 69$) with upfront HID-HSCT ($n = 89$) in SAA patients [14]. There was a higher prevalence of grade II–IV aGVHD (30.3% vs. 1.5%, $p < 0.001$) and cGVHD (30.6% vs. 4.4%, $p < 0.001$) in the HID group. However, the 3-year OS (86.1% vs. 91.3%) and FFS (85.0% vs. 89.8%) rates were comparable. The donor source did not affect the primary engraftment and survival outcomes. However, other researchers have shown contradictory results. Zeng et al. [15] reported that the incidence of grade II–IV aGVHD and cGVHD were not significantly different between the HID and MRD groups (34.5% vs. 25.7%; 18.5% vs. 28.8%). The OS rate in patients > 40 years old in the HID group was significantly inferior to that in those of the MRD group (37.5% vs. 83.3%, $p = 0.02$). In the report by Li et al. [16], patients of the HID group had a lower OS rate than those in the MRD group (75.0% vs. 82.8%, $p = 0.029$). Ferritin levels and infections were significant risk factors for OS by the multivariate analyses. In young patients, compared with the matched unrelated donor (MUD) cohort, the HID cohort had an increased

Table 1 The clinical outcome of HID-HSCT in China

Author [Ref.] (year)	Years of treatment	Patient, no	Age median (range)	Graft failure, no. (%)	GVHD, %		Infection %		Survival. %		Follow-up, median (range), months
					Acute	Chronic	CMV	EBV	OS	FFS	
Xu et al. [6] (2017)	2007–2015	HID 52	9 (2–17)	3 (5.9)	39.2 II–IV	34.2	69.2	5.8	84.5 3 years	82.7 3 years	24.8 (3.3–109.8)
Xu et al. [7] (2018)	2011–2016	HID 51	25 (18–45)	0	20.0 II–IV	25.9	84.0	16.0	83.5 3 years	83.5 3 years	21.1(3.2–71.1)
Xu et al. [8] (2019)	2009–2017	HID 76	28 (18–49)	2 (2.7)	25.3 II–IV	21.3	na	na	83.7 8 years	83.7 8 years	24.7 (6.1– 103.0)
		IST 37	32 (18–62)						75.6 8 years	38.5 8 years	20.2 (3.2–96.0)
Yang et al. [9] (2019)	2012–2016	HID 51	13(4–18)	0	40.0 II–IV	15.0	65.0	15.0	85.0 3 years	80.0 3 years	28.9 (0.5–47.1)
		IST 29	12(4–17)						79.3 3 years	35.9 3 years	51.8 (0.5–81.8)
Xu et al. [12] (2017)	2012–2015	HID 89	22 (4–51)	1(1.1)	30.3 II–IV	30.6	51.7	28.1	86.1 3 years	85.0 3 years	21.4 (7.1–47.6)
		MRD69	33 (7–61)	1(1.5)	1.5 II–IV	4.4	43.5	21.7	91.3 3 years	89.8 3 years	26.0 (7.5–47.6)
Zeng et al. [13] (2018)	2006–2015	HID115	18 (3–58)	12(10.4)	34.5 I–IV	18.5	35.6	na	74.8 5 years	na	20.4 (1–102)
		MRD74	27(3–53)	3(4.1)	25.7 I–IV	28.8	15.3	na	78.1 5 years	na	
Li et al. [14] (2018)	2007–2016	HID116	19 (2–52)	7 6.4)	46.6 I–IV	27.0	na	na	75.0 3 years	na	24 (0–118)
		MRD119	26 (6–56)	10 (8.6)	12.6 I–IV	7.2	na	na	82.9 3 years	na	40 (0–128)
Lu et al. [15] (2018)	2012–2016	HID41	13 (4–42)	0	43.9 II–IV	59.8	65.9	26.8	80.3 3 years	76.4 3 years	25 (6–45)
		MUD 48	9 (2–28)	0	12.5 II–IV	47.6	60.4	18.7	89.6 3 years	89.4 3 years	24 (4–46)
Liu et al [16] (2018)	2011–2017	HID + UCBT127	23(3–54)	1(0.8)	24.8 II–IV	37.3	na	na	78.5 4 years	77.4 4 years	20.5 (4–60)
Liu et al [17] (2017)	2013–2015	HID + MSCs 44	24 (8–47)	1(2.4)	29.3 II–IV	14.6	65.9	31.8	77.3 1 year	na	12 (0.9–30.8)
Wang et al. [18] (2018)	2014–2016	HID + MSCs 35	11.5(3–18)	0	25.7 II–IV	22.3	31.43	48.57	85.7 3 years	na	22 (3.5–37)

HID-HSCT haploidentical donor hematopoietic stem cell transplantation, *MRD* matched related donor, *MUD* matched unrelated donor, *IST* immunosuppressive treatment, *OS* overall survival, *FFS* failure-free survival, *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *UCBT* unrelated cord blood transplantation, *MSCs* mesenchymal stem cells, *na* not available

CI of grades II–IV aGVHD (43.9% vs. 12.5%, $p=0.001$); however, they had similar CI of limited cGVHD (47.7% vs. 38.5%), extensive cGVHD (12.1% vs. 9.1%), and 3-year OS (80.3% vs. 89.6%) [17].

To improve the result of HID-HSCT, Liu et al. tried to combine HID-HSCT with an unrelated cord blood unit in 127 AA patients. Except 1 patient, all achieved complete haploidentical engraftment. The median durations of myeloid engraftment were 11 (9–28) days and 15 (9–330) days for platelets. The incidence of grade II–IV aGVHD and cGVHD was 24.7% and 14.1%, respectively. The 4-year OS rate was 78.5% [18]. This strategy may be feasible choice for SAA patients.

MSCs have been shown to support hematopoiesis in vivo and display potent immunosuppressive effects for treating GVHD after HSCT. Donor-BM derived MSCs were co-transplanted following HID-HSCT. A multicenter phase II trial has shown promising results. Forty of the 41 patients (97.6%) achieved engraftment and sustained full donor chimerism. The incidence was 29.3% for grade II–IV aGVHD and 14.6% for cGVHD. The OS rate was 77.3% with a median follow-up duration of 1 year [19]. A low incidence of graft failure, aGVHD, and cGVHD has also been reported in a pediatric SAA population [20].

As an upfront and salvage therapy, HID-HSCT is an effective and safe option for SAA patients, with outcomes comparable to those of IST, MRD-HSCT,and MUD-HSCT. HID-HSCT could be evaluated as a treatment option for SAA patients < 40 years old in countries other than China.

Porcine anti-human lymphocyte immunoglobulin (pALG)

Today, the standard immunosuppressive regimen is a combination of ATG and CyA. ATG that originates either from horse or rabbit serum is commonly used worldwide. However, horse ATG is unavailable in China. pALG and rabbit ATG (rATG) are the two products used for SAA treatment in China. pALG was approved by the Sino Food and Drug Administration in 2004. It is a polyclonal antibody obtained from pigs immunized with human T lymphocytes. In 2010, Han et al. first reported 48 SAA patients treated with pALG and CyA. The overall response (OR) was 83.3% [54.2% CR, 29.2% partial response (PR)] with a median time of 90-days from the start of IST (range 23–380 days). Mild toxicities, including acute allergic reaction and elevated liver function, were observed in half of the patients. The actuarial OS at 1.5 years was 87.5% [21]. Similar efficacy and safety profiles could also be found in Wei's research [22]. Chen et al. conducted a long-term follow-up study of pALG and CyA in 102 SAA patients. The OR was 79.4% (CR 22.5%, PR 56.9%) at 6 months. The 5-year OS rate after treatment was 81.8% (95% CI; 74.2–90.2%) with a median follow-up duration of 56.9 months. Clonal evolution occurred in 7.8% of the patients, including 4.9% of symptomatic paroxysmal nocturnal haemoglobinuria (PNH) [23]. High efficacy without serious adverse effects was reported in a study on pediatric SAA patients [12] (Table 2).

In the study of a comparison between pALG ($n=43$) and rATG ($n=32$) as the first-line treatment [24], there were no significant differences in the OR of the pALG and rATG groups at 3 months (41.8% vs. 40.6%), 6 months (66.6% vs. 61.2%) and 2-year OS (87.4% vs. 83.2%). Further, no difference was found in the blood recovery: neutrophil count to reach $\geq 1\times 10^9$/L, independence of RBC transfusion and platelets transfusion (21 days vs. 19 days, 57 days vs. 60 days, 49 days vs. 52 days, respectively). The incidences of toxicities in the pALG and rATG groups were comparable, including serum sickness (18.6% vs. 21.8%) and liver dysfunction (23.2% vs. 21.8%). The incidences of relapse and clonal evolution were also similar between the pALG and rATG groups (4.6% vs. 6.2% and 4.6% vs. 3.1%). Chen et al. retrospectively compared the outcomes in 148 patients with SAA who received pALG ($n=114$) or rATG ($n=34$) as an upfront therapy. The pALG group achieved a better OR than the rATG group at 3 months (65.8% vs. 44.1%, $p=0.023$) and a lower incidence of myelodysplastic syndromes (MDS)/acute myeloid leukemia (AML) transformation (2.6% vs. 11.8%, $p=0.049$). No significant difference in the OS or FFS rates was observed between the pALG group and the rATG group. The 5-year OS rate was 80.5% for the pALG group and 74.1% for the rATG group (Table 2). The lymphocyte counts of the rATG group remained significantly lower than those of the pALG group after 1, 3, and 6 months from treatment initiation and recovered to equivalent levels after 12 months [13]. When compared the changes in subsets of T cell, the inhibitory effects on the CD8 + T cells were not different between these two groups, while the inhibitory effects on CD4 + T cells were significantly higher in the rATG group than in the pALG group [25]. rATG demonstrated stronger immunosuppressive effects than pALG. However, the stronger immunosuppressive effect did not reflect the efficacy of IST. For older people, in Li's research [26] with median age 63.5 (60–79) years, two (22%) of the 9 patients treated with rATG achieved hematological response; in contrast, all 7 patients (100.0%) treated with pALG achieved hematological response. pALG was better than rATG as a first-line treatment for older patients with SAA.

In sum, pALG + CyA showed similar or superior efficacy to rATG + CyA as a first-line treatment for SAA. Compared to rATG, pALG is cheaper and lowers the cost of therapy by 30–50%. Considering the advantage of lower cost, it is reasonable to use pALG rather than rATG.

Table 2 The clinical outcome of pALG trials in China

Author [Ref.] (year)	Years of treatment	Age, median (range)	ATG	no	6 months			Final follow-up			OS (%)	Follow-up, median (range) months	Relapse no. (%)	PNH transformation no. (%)	MDS/AML transformation no.(%)
					PR%	CR%	OR%	PR%	CR%	OR%					
Han et al. [21] (2010)	1999–2009	28 (13–64)	pALG	48	na	na	na	29.2	54.2	83.3	87.5 1.5 years	18 (2.9–60.8)	1 (2.5)	na	na
Wei et al. [22] (2015)	2009–2013	27(14–52)	pALG	69	na	na	73.9	20.3	56.5	76.8	88.4 2 years	24 (4–44)	1 (1.4)	na	1 (1.4)
Liu et al. [24] (2015)	2005–2013	22 (3–64)	pALG	43	44.1	22.6	66.7	na	na	na	87.4 2 years	29 (5–103)	2 (4.7)	2 (4.7)	na
		23 (3–70)	rATG	32	42.2	19.1	61.3	na	na	na	83.2 2 years	28 (3–100)	2 (6.3)	1 (3.1)	na
Chen et al. [23] (2016)	1999–2014	29 (12–72)	pALG	102	56.9	22.5	79.4	32.4	42.1	74.5	81.8 5 years	59.6 (0.2–176.8)	8 (9.9)	5 (4.9)	2 (1.9)
Ma et al. [25] (2016)	2004–2013	21 (4–56)	pALG	45	68.4	7.9	76.3	56.8	27.0	83.8	82.2 5 years	31.5 (16.1–58)	32 (6.7)	1 (2.2)	1 (2.2)
		25(7–64)	rATG	32	37.0	11.1	48.1	25.9	40.7	66.6	68.8 5 years	54 (12.9–60.8)	na	na	na
Cui et al. [12] (2018)	2003–2011	14(5–17)	pALG	50	na	na	64	na	na	na	80 10 years	59.6 (11–176.8)	5 (10)	na	3 (6)
Chen et al. [13] (2018)	1996–2015	30(12–72)	pALG	114	49.1	25.4	74.6	na	na	na	80.5 5 years	70.3 (6.1–138.5)	10 (8.8)	6 (5.3)	3 (2.6)
		35(14–76)	rATG	34	41.2	23.5	64.7	na	na	na	74.1 5 years	29.9 (9.1–158.6)	2 (5.9)	1 (2.9)	4 (11.8)

pALG porcine anti-human lymphocyte immunoglobulin, *rATG* rabbit anti-human thymocyte immunoglobulin, *PNH* paroxysmal nocturnal haemoglobinuria, *MDS* myelodysplastic syndromes, *AML* acute myeloid leukemia, *PR* partial response, *CR* complete response, *NR* no response, *OR* overall response = PR + CR, *OS* overall survival, *na* not available

Mesenchymal stromal cells

Impairments of the BM microenvironment can contribute to defective hematopoiesis in SAA patients. MSCs are a major component of the hematopoietic microenvironment, and several studies have reported abnormal function of MSCs in SAA patients [27–29]. BM-MSCs also exert immunomodulatory effects on activated lymphoid cells, including T cells, B cells, natural killer cells, and dendritic cells [30]. Thus, several study groups in China have attempted intravenous administration of MSCs from a related donor to treat patients with refractory SAA. After a follow-up period of 1 year, 6 of the 18 patients achieved a complete or PR without any serious adverse events related to the MSC transfusion. An increased proportion of CD4 + CD25 + FOXP3 + regulatory T cells in the peripheral mononuclear cells were observed in all 18 patients [31]. Pang et al. conducted a phase II study in SAA patients refractory to IST. Seventy-four patients received allogeneic BM-MSCs at a dose of $1–2 \times 10^6$ cells/kg per week for 4 weeks. The OR rate was 28.4% with 6.8% CR and 21.6% PR. The median times to response of leukocytic, erythrocytic, and megakaryocytic lineages were 19 (11–29), 17 (12–25), and 31 (26–84) days, respectively. After a median follow-up of 17 months, the OS rate was 87.8%. Three patients progressed to myelodysplasia, and 2 developed clonal cytogenetic abnormalities (1 patient developed chromosome 7 abnormalities, while the other had a complex karyotype). The use of ATG in previous treatment and absence of infections throughout the treatment period were predictors of a favorable response. The authors concluded that allogeneic BM-MSCs infusion is a feasible and effective treatment option for refractory SAA [32]. Considering the advantages of safety, lower costs, and rapid response, MSCs can be considered in the first- line treatment of SAA.

Treatment algorithm for SAA

The Guidelines of the British Society for Hematology list MRD-HSCT and MUD-HSCT as the first-line standard cares for pediatric SAA patients [33]. In adult SAA patients, MUD-HSCT should be considered in cases of lack of response to 1 course of IST. HID-HSCT and unrelated cord blood transplantation are still in their experimental stages; therefore, they should only be offered by centers experienced in these approaches within clinical protocol studies [34]. However, indications of HSCT for SAA are not strictly separated as per donor source in China. According to the latest recommendations of the Chinese Society of Hematology, HID-HSCT can be considered a front-line treatment for young patients without a suitable MRD or MUD [35].

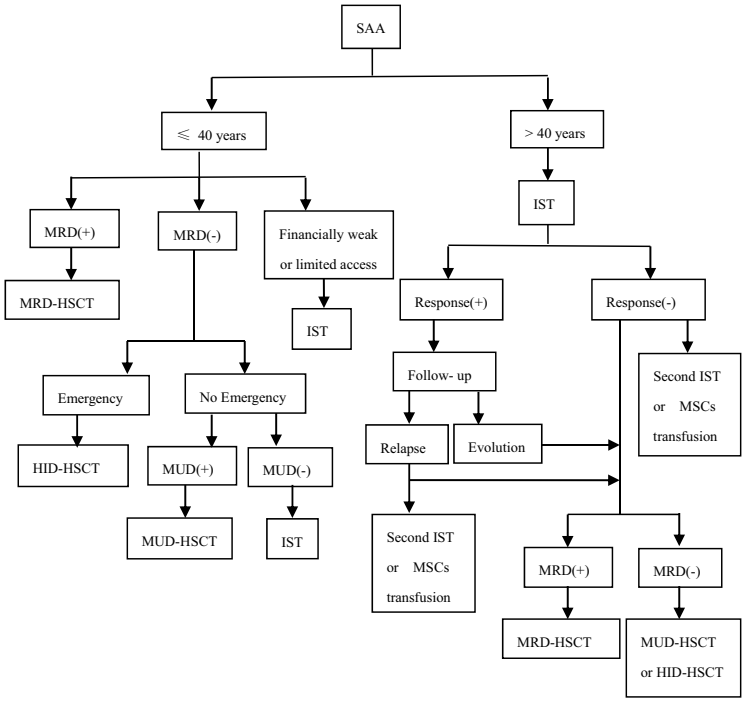
In this review, the OS and FFS rates of HID-HSCT were comparable with those of MRD-and MUD-HSCT. HID-HST showed a comparable OS and better FFS rate than IST in patients < 40 years old [8, 9, 14–17]. All patients eligible for HSCT are recommended to undergo HLA typing at the time of diagnosis, followed by MRD or MUD searches to assess the availability of potential donors. aGVHD and cGVHD have a lower incidence in MRD-HSCT; therefore, MRD-HSCT is recommended as a first- line treatment when a MRD is available. If a MRD is unavailable, and provided a suitable MUD can be identified rapidly, MUD-HSCT could become the upfront therapy. HID-HSCT should be regared as a salvage therapy when young patients fail to respond to IST. For emergent cases, HID-HSCT can be considered as a front-line treatment, when a MRD is not available. However, before we get solid data on the long-term effects of the conditioning regimens, it is too early to generalize the recommendations in every centers. For financially weak patients or those who have limited access to medical facilities that perform HSCT, IST with ATG and CyA is still indicated as the first-line therapy.

IST is also recommended as a first-line therapy for patients aged > 40 years. A second course of ATG or MSCs transfusion may be indicated in case of lack of response or relapse following the first course. MRD-HSCT (if a MRD is available) or MUD-HSCT and HID-HSCT are recommended as the second-line treatment in cases of failure, relapse, or evolution after 1 course of IST. (Fig. 1).

The standard immunosuppressive regimen is a combination of ATG and CyA. ATG is obtained by immunizing animals with cells from fetal thymic tissues. Owing to the different species of the immunized animals, ATG currently encompasses hATG, rATG, and pALG. The combination of hATG with CyA as a first-line treatment of SAA achieves a hematological response of 60–75% [36], while that with rATG as a second-line treatment achieves an OR of 30–77% [37, 38]. However, rATG appeared less effective than hATG when used as a first-line IST [39]. The OR of pALG reached 66.6–83.8%.[12, 13, 21–25]. pALG showed similar or superior efficacy compared to rATG as a first-line treatment [13, 24–26]. However, hATG is unavailable in China; therefore, there are no data to compare pALG with hATG. The combination of pALG and CyA is recommended as an IST that offers the advantages of equal efficacy and lower cost as compared to rATG for SAA patients.

Conclusion

The published evidence from China suggests that MRD-HSCT and MUD-HSCT can be recommended as a first-line treatment for pediatric and young-adult SAA patients.



SAA:severe aplastic anemia; HSCT:hematopoietic stem cell transplantation; MRD:matched related donors; MUD: matched unrelated donor HID: haploidentical donor; IST: immunosuppressive treatment; MSCs:mesenchymal stromal cells.

Fig. 1 Treatment algorithm for severe aplastic anemia

HID-HSCT should be considered as a salvage therapy when young patients fail to respond to IST. pALG can replace rATG as an immunosuppressive drug. BM-MSCs infusion can be used as a second-line treatment for refractory SAA. Further studies with larger cohorts and longer follow-up periods are warranted to establish a refined algorithm for SAA treatment.

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Author contributions SK had the idea for the article, LY performed the literature search and data analysis, and FKZ and SK drafted and/or critically revised the work.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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·综述·

猪源抗人淋巴细胞球蛋白治疗再生障碍性贫血的研究进展

张会勤　王化泉　邵宗鸿

Progress in the treatment of severe aplastic anemia with porcine anti-human lymphocyte globulin　*Zhang Huiqin, Wang Huaquan, Shao Zonghong*

Corresponding author: Shao Zonghong, Department of Hematology, General Hospital, Tianjin Medical University, Tianjin 300052, China. Email: shaozonghong@sina.com

再生障碍性贫血(AA)是一种获得性骨髓造血功能衰竭综合征,主要表现为骨髓造血功能低下、全血细胞减少和由此导致的贫血、出血、感染症状。近年来的研究证实,细胞毒性T淋巴细胞(CTL)及其分泌细胞因子功能亢进,导致造血干/祖细胞免疫损伤是AA发病的主要机制[1-2]。根据疾病严重程度,AA可分为极重型AA(VSAA)、重型AA(SAA)、非重型AA(NSAA)。免疫抑制治疗(IST)和同胞HLA相合骨髓造血干细胞移植(HSCT)是目前治疗AA的两种主要方法[3-4]。抗人胸腺/淋巴细胞球蛋白(ATG/ALG)和环孢素A(CsA)是IST的主要药物;同时,ATG/ALG也是SAA患者HSCT预处理方案中的重要药物。ATG/ALG分为马源、兔源和猪源。马源ATG在美国外的大部分国家无供应。

猪源ALG(p-ALG)是我国自主研制的药物,广泛应用于临床多年,治疗SAA取得了较理想疗效,费用相对低廉,使用安全。现我们现对p-ALG治疗AA的作用机制、疗效和安全性作一综述。

一、p-ALG的研发和制备

p-ALG由武汉生物制品研究所于20世纪70年代开始研制,1983年获得生产批号进行生产。开始主要采取硫酸铵盐析法,因为产量低、损失大、高污染、成本高、过程复杂和周期长等缺点,后改为低温乙醇法,具有工艺稳定、安全无毒、产量高、低污染等优势。制备的流程[5-6]:人外周血T淋巴细胞或人胸腺细胞免疫健康猪,猪血采集、分离血浆,应用低温乙醇法制备免疫球蛋白,然后进行免疫球蛋白的精制,健康人血红细胞吸收红细胞抗体,健康人胎盘渣吸收抗血小板抗体,健康人混合血浆吸收抗人血浆蛋白抗体,免疫球蛋白的病毒灭活,半成品的检定,分装及成品的检定。由于历史原因,2004年该药重新获中国食品和药品管理局批准。

余建等[5]研究低温乙醇法工艺生产p-ALG,通过E玫瑰花环形成抑制试验及淋巴细胞毒试验效价测定ALG制品,经生化检测,该制品纯度不低于蛋白质总量的90.0%。IgG单体与二聚体含量之和不低于90.0%,多聚体含量低于5.0%。E玫瑰花环形成抑制试验效价不低于1∶4 000。淋巴细胞毒试验效价不低于1∶1 000。人红细胞抗体效价低于1∶64。血小板抗体效价低于1∶4。人血浆蛋白抗体试验为阴性。

张颂等[7]研究低温乙醇法工艺生产的p-ALG的有效期。p-ALG置(6±2)℃贮存,分别于0、3、6、9、12、18、24、30、36个月取样,对制品外观、pH、纯度、蛋白含量、分子大小分布以及效价进行检定。E玫瑰花环形成抑制试验及淋巴细胞毒试验计算获得有效期。结果根据E玫瑰花环形成抑制试验效价测定结果,理论推算在(6±2)℃条件下保存时,有效期可达41~100个月;根据淋巴细胞毒试验效价测定结果,理论推算在(6±2)℃条件下保存时,有效期可达47.9~64.3个月。结论初步确定p-ALG制品有效期为30个月。

夏南等[8]应用高效液相色谱测定p-ALG中戊二醛残留量,2批供试品中戊二醛的含量分别为9.8和7.5 μg/ml,检测5、10、15 μg/ml戊二醛的回收率分别为102.3%、101.6%和99.2%。回收后样本中戊二醛无残留。

詹合琴等[9]观察p-ALG对小鼠的急性毒性作用。分别采用腹腔注射限度试验和尾静脉注射最大受试药量试验法,连续观察14 d后处死、解剖小鼠,肉眼观察发现异常的组织或器官进行病理切片检查。所有试验动物存活,供试品的半数致死剂量(LD50)>5 g/kg,但尾静脉注射最大给药量试验时发现给药2 d内动物的一般情况较差,处死并解剖后发现用药组有少数小鼠出现肝、肾的感染性侵害。p-ALG的LD50 >5 g/kg(相当于临床用量的250~333倍),提示该药物比较安全,但在过量静脉注射后短期内对小鼠仍有一定毒性。詹合琴等[10]进一步应用p-ALG治疗AA小鼠的结果显示,p-ALG能明显改善AA小鼠骨髓造血功能,有核造血细胞数量明显增多。

二、p-ALG治疗AA的作用机制

AA发病机制包括造血干细胞内在增殖缺陷、造血微环境支持功能缺陷、免疫调节功能紊乱及遗传倾向等。越来越多的证据显示,CTL及其分泌细胞因子　IFN-γ和TNF-α功能亢进和调节T细胞不足,导致造血干/祖细胞免疫损伤和骨髓微环境异常是AA发病机制的主要环节[11-12]。目前国内、国外关于ATG/ALG作用机制的报道多为马源和兔源制剂,p-ALG的作用机制尚未阐明。

三、p-ALG联合CsA治疗SAA的研究现状

1. 剂量和用法:p-ALG为每支250 mg,一般使用剂量为20~30 mg·kg^{-1}·d^{-1},稀释于250~500 ml 0.9%氯化钠注射液中

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作者单位:300052　天津医科大学总医院血液科

通信作者:邵宗鸿,Email:shaozonghong@sina.com

静脉滴注，开始速度5~10滴/min，若10 min后无反应，再逐渐加速，全量在1~2 h内输注完，连用5 d。使用前需进行皮试，皮试阴性者方可使用。输注时，同时建立另一条静脉通路滴注肾上腺糖皮质激素（等效于泼尼松1 mg·kg^{-1}·d^{-1}×5 d）预防过敏反应及血清病，后序贯改用口服泼尼松1 mg·kg^{-1}·d^{-1}，逐渐减量，第30天停用，根据有无血清病调整剂量。

2. 疗效：p-ALG治疗SAA的总有效率（ORR）为65%~80%[13-21]。北京协和医院韩冰教授团队首先报道了p-ALG治疗大样本SAA的疗效。回顾性分析了从1999年至2009年48例联合应用p-ALG（20~30 mg·kg^{-1}·d^{-1}，第1~5天）和CsA（3~5 mg·kg^{-1}·d^{-1}）治疗SAA患者。48例患者ORR为83.3%，54.2%基本治愈，29.2%缓解，6.3%无效。治疗起效的中位时间为90（23~380）d。基本治愈组患者脱离输血的中位时间为70（23~280）d，明显短于缓解组患者的180（95~380）d（*P*=0.002）。治疗至脱离输血时间的中位数为99 d，上下四分位数分别为55及275 d。治疗后1.5年完全脱离输血的患者占96%，1.5年的总生存（OS）率为87.5%[13-14]。随后进一步扩大样本，共纳入102例SAA患者，验证p-ALG联合CsA治疗SAA的远期疗效：中位随访59.6（0.2~176.8）个月，IST治疗3个月后，ORR为71.6%；6个月后，ORR为79.4%，5年OS率为81.8%（95%*CI* 75.5%~93.2%）[15]。

Wei等[16]报道了应用p-ALG联合CsA治疗69例SAA的结果，中位随访时间为24（4~44）个月，ORR为76.8%，完全血液学反应率达56.5%，部分血液学反应率为20.3%。中位起效时间90 d。3、6、12、18和24个月ORR分别为63.8%、73.9%、76.8%、75.4%和75.4%。2年OS率为88.4%。2年无病生存率为85.5%。

多个研究[17-20]均回顾性分析比较了p-ALG和兔源ATG（r-ATG）治疗初治SAA的疗效，结果显示p-ALG和r-ATG组中性粒细胞绝对计数（ANC）≥1×10^9/L、脱离红细胞输注时间、脱离血小板输注时间无明显不同；且p-ALG联合应用CsA、G-CSF等后较单用药物治疗获得更好的疗效，r-ATG组也是这个规律；p-ALG治疗SAA和VSAA的ORR组间差异无统计学意义，与r-ATG组一致。

此外，有相关研究报道p-ALG和r-ATG作为移植前预处理药物之一的疗效比较。陈育红等[22]分析比较p-ALG与r-ATG分别在非血缘关系骨髓移植与非血缘关系外周造血干细胞移植的预处理的疗效，OS率分别为47.22%和62.45%（*P*=0.114）。对两组ATG的来源进行分层分析后，两组急性、慢性移植物抗宿主病（GVHD）的发生率和严重程度差异均无统计学意义。Ye等[23]报道异基因HSCT联合中药治疗11例SAA患者，以p-ALG作为移植前预处理药物，5年的OS率为81.8%。Ⅰ~Ⅱ度、Ⅲ~Ⅳ度急性GVHD发生率分别为18.2%、9.1%；慢性GVHD发生率为27.3%。

Tang等[24]报道ALG/ATG联合中药治疗27例VSAA患者，16例使用p-ALG，5例使用马源ATG，6例使用r-ATG，27例患者1年的ORR为80%，36.0%基本治愈，20%缓解，24%明显改善，20%无效。

3. 疗效预测因素：Han等[13]报道了年龄、性别、病情危重程度、诊断至治疗间隔时间、治疗前合并感染对ORR没有影响。而Wei等[16]报道高龄、VSAA和淋巴细胞数低是预后差的指标。Ma等[19]对比后发现只有VSAA、诊断至治疗间隔时间≥30 d、治疗6个月无反应为预后不良指标。VSAA组5年内死亡风险高于SAA组1.5倍（*P*=0.04，95% *CI* 1.03~5.68），诊断至治疗间隔时间≥30 d患者死亡风险比诊断至治疗间隔时间<30 d患者高2.6倍（*P*=0.01，95% *CI* 1.50~10.10），治疗6个月无反应患者死亡风险比有反应患者高2.75倍（*P*=0.01，95% *CI* 1.03~6.04），该结果与Liu等[17]的报道相似。杨云等[20]报道示病程≥3个月及外周血ANC<0.2×10^9/L患者的疗效明显低于病程<3个月及外周血ANC≥0.2×10^9/L的患者。

4. 早期病死率：Han等[13]报道了48例患者30 d内的早期病死率为10.4%；扩大样本纳入102例SAA患者报道早期病死率为16.7%，中位死亡时间2.3（0.2~36）个月[15]。Wei等[16]报道60 d内总体病死率为10.1%（7/69）。Ma等[19]对比p-ALG组和r-ATG组早期病死率差异无统计学意义，而治疗5年内病死率分别为15.56%与25%（*P*=0.302）。由此可知p-ALG治疗SAA早期病死率一般在10%~20%。杨云等[20]报告的早期病死率为11.1%（4/36）。患者死亡原因包括感染、脑出血、缺血性心脏病和进展为其他血液病，最常见的死亡原因为感染。所以在治疗的过程中应当注意预防感染等并发症，以提高有效率、改善预后。

5. 克隆性演变：Han等[13]未报道48例患者演变为克隆性疾病。而该研究扩大样本、中位随访时间延长至59.6（0.2~176.8）个月后发现7.8%出现克隆演变，其中阵发性睡眠性血红蛋白尿症（PNH）5例，骨髓增生异常综合征（MDS）2例[15]。Wei等[16]、杨云等[20]各报道1例患者出现克隆进展，分别进展为MDS与MDS-难治性贫血伴有原始细胞过多（RAEB）。因此，在p-ALG治疗SAA的过程中，有发生克隆演变为PNH、MDS、MDS-RAEB等其他血液学疾病，应定期检测。

6. 复发：Han等[13]、Wei等[16]报道在随访期内均有1例患者出现复发；随后Chen等[15]扩大样本后的复发率为9.9%。Liu等[17]报道p-ALG组和r-ATG组复发率为4.65%和6.25%，而Ma等[19]报道p-ALG组复发率为6.67%，r-ATG组无复发病例。总体来说，应用p-ALG治疗复发率低。

7. 不良反应：Han等[13]报道p-ALG治疗48例SAA患者出现发热25例（52.1%），伴皮疹，应用激素及对症治疗后可恢复正常；血清病8例（16.7%）；肝功能损伤29例（60.4%）；出血1例（2.1%）；未出现肌酐升高、过敏性休克及因p-ALG并发症死亡病例。随着样本量的扩大及随访时间的延长，常见不良反应仍是输注过敏反应和血清病反应[22.5%（23/102）]，包括发热、皮疹、关节疼痛，2例出现自身免疫性溶血性贫血，其中1例经糖皮质激素治疗后好转，1例治疗后出现肺部感染死亡[15]。中性粒细胞缺乏常导致感染，血小板

减少可以出现严重出血。而Wei等[16]报道p-ALG治疗SAA患者33.3%出现过敏反应，21.7%出现血清病反应，27.5%出现肝功能异常。Liu等[17]、Ma等[19]对比后报道p-ALG和r-ATG两组的不良反应相似，上述不良反应经过合理的对症处理后可得到控制，且患者多能耐受。

四、小结

国产p-ALG具有自主知识产权，临床疗效良好，不良反应低。但p-ALG治疗SAA多为单中心、非随机临床试验，如果要将该药推广至国外，纳入SAA的标准治疗方案，仍需要进行多中心、随机对照临床试验研究。最佳治疗方案也有待于进一步探讨。p-ALG治疗SAA的机制也有待于进一步研究。

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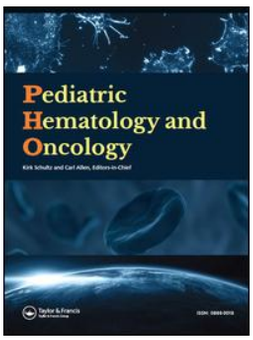

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Efficacy and safety of immunosuppressive therapy with or without eltrombopag in pediatric patients with acquired aplastic anemia: A Chinese retrospective study

Meixin Fang, Hua Song, Jingying Zhang, Sisi Li, Diying Shen, and Yongmin Tang

Department of Hematology-Oncology, Pediatric Hematology-oncology Center, The Children's Hospital of Zhejiang University School of Medicine, National Clinical Research Center For Child Health, Hangzhou, PR China

ABSTRACT

To determine the efficacy and safety of eltrombopag (E-PAG) combined with intensive immunosuppressive therapy (IST) for the treatment of pediatric patients with severe aplastic anemia (SAA). A total of 57 pediatric patients with newly diagnosed severe aplastic anemia were enrolled in this study. Thirty nine patients were treated with IST alone, consisting of porcine anti-human thymocyte globulin (30 mg/kg/day × 5 days) and cyclosporine A (CsA) (treated for 2 years, with a trough concentration maintained at 200–250 ng/mL), and 18 patients were treated with IST + E-PAG (12.5–50 mg/day, maintained for 6 months). We found no statistical difference between the response rates at 3 months for the two groups (CR: 12.8% vs. 22.2% $p > 0.05$, ORR: 56.4% vs. 77.7% $p > 0.05$). However, we found a statistical difference between the response rates at 6 months for the two groups (CR: 17.9% vs. 50% $p < 0.05$, ORR: 69.2% vs. 94.4% $p < 0.05$). The main side-effect during treatment with E-PAG was having a slightly to moderately elevated bilirubin level, which was temporary and controllable, accounting for approximately 66.6% (12/18) of patients in the IST + E-PAG group vs. 20.5% (8/39) of those in the IST group ($p < 0.05$). IST + E-PAG therapy appears to be more effective than IST alone for the treatment of pediatric SAA, with good tolerability and compliance. This approach deserves further exploration.

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1. Introduction

Severe aplastic anemia (SAA), a significant threat to human health, manifests as peripheral hemopenia due to the immune-mediated destruction of bone marrow cells.[1,2] The main pathogenic mechanism of SAA is the abnormal activation and hyperfunction of T lymphocytes, leading to the immune destruction of hematopoietic stem cells (HSCs).[3] SAA presents mainly as anemia, bleeding, infections, and other clinical manifestations of pancytopenia. The majority of those patients that did not respond to immunosuppressive therapy (IST), succumbed to infections or bleeding.[4]

CONTACT Yongmin Tang y_m_tang@zju.edu.cn Department of Hematology-Oncology, Pediatric Hematology-oncology Center, The Children's Hospital of Zhejiang University School of Medicine, National Clinical Research Center For Child Health, # 57 Zhuganxiang Road, Yan-an Street, Hangzhou 310003, PR China.

The current treatment approach for SAA consists of IST and hematopoietic stem cell transplantation (HSCT). Anna et al. found that in a study of 607 children who received bone marrow transplantation (BMT) and 307 who received IST, the overall survival rates were 83% for matched sibling donors (MSD) BMT and 81% for IST, respectively.[5] IST is an effective treatment method for patients with SAA if there is no suitable donor or for those who are >35–50 years of age.[6] The standard immunosuppressive treatment protocol involves the use of the anti-thymocyte globulin (ATG) and cyclosporine A (CsA). Some studies have found that horse ATG was superior to rabbit ATG with respect to short-term response and long-term survival[7,8]; other studies have also shown that horse ATG was more effective than rabbit ATG for treating AA in children.[9,10] However, in some countries, including China, horse ATG is not available.[11] Therefore, we have been using porcine ATG (pATG) to treat patients with SAA since the 1980s. pATG is comparable to other ATG formulations in terms of short- and long-term efficacy and safety.[12]

Scheinberg et al found that the overall response rate (ORR) and complete response rate (CRR) for IST at 6 months in children with SAA were 74% and 26%, respectively.[13] Some studies have also shown similar results for IST in children with SAA.[14,15] While these findings were supported by other studies that reported better response rates,[16,17] there still remained patients who were unresponsive to IST. A possible reason for the poor immunosuppressive effect in this group of patients may be due to a more severe immune-mediated destruction of HSCs.[18]

It has been reported that thrombopoietin (TPO) can stimulate the hematopoietic capacity of primitive HSCs in the bone marrow.[19,20] Thus, theoretically, TPO analogues can effectively improve the bone marrow function of SAA patients. As an agonist of the TPO receptor, eltrombopag (E-PAG) has been found to significantly restore trilineage hematopoiesis in patients with refractory aplastic anemia (AA), which can be sustained even on discontinuation of the drug.[21–24] Jin et al also found that the complete and overall response rates of hematologic response among Chinese adult patients with severe aplastic anemia treated with IST + E-PAG was higher than those without E-PAG.[25] However, the effects of E-PAG on patients with SAA remain controversial. In elderly patients with SAA, the combination of E-PAG with ATG + CsA has shown good tolerability and significant therapeutic efficacy,[26] while the addition of E-PAG to IST for children with AA did not improve the ORR at 6 months.[27] Another study in three children receiving E-PAG therapy showed one complete response (CR), one partial response (PR) and one no response (NR).[17] Therefore, we conducted a retrospective study of 57 cases in order to determine the safety and efficacy of E-PAG combined with IST in pediatric patients with SAA.

2. Methods

2.1. Study design and patient selection

This was a single-center, retrospective cohort study that assessed the safety and efficacy of E-PAG in immunosuppressive therapy for pediatric patients with SAA between January 2012 and October 2018. The data of these patients during this period were reviewed retrospectively. This study was approved by the Ethical Committee of the

Children's Hospital of Zhejiang University School of Medicine, National Clinical Research Center for Child Health (No. 2018-IRBAL-068). Appropriate informed consents were obtained from all patients and/or their legal guardians according to the Declaration of Helsinki.

A total of 57 pediatric patients with SAA were enrolled in this study. For the period from January 2012 to December 2016, 39 patients were treated with only IST. During that period, E-PAG had not yet been introduced into our hospital. After the State Food and Drug Administration of China approved the use of E-PAG and with encouraging results from its use in adult patients with SAA, 18 consecutive pediatric patients were treated with IST + E-PAG from January 2017 to October 2018.

According to the Camitta criteria, severe aplastic anemia (SAA) was defined as a bone marrow cellularity of 25% $\sim$ 50% and less than 30% residual hematopoietic cells plus at least 2 of the following parameters: an absolute neutrophil count (ANC) $<0.5 \times 10^9$/L, platelet count $<20 \times 10^9$/L, or absolute reticulocyte count (ARC) $<20 \times 10^9$/L. In addition to meeting the SAA criteria, very severe aplastic anemia (VSAA) was defined as an ANC below 0.2×10^9/L.

The study inclusion criteria were as follows: (1) newly diagnosed patients with treatment-naïve SAA and (2) children aged $\leq$16 years old. The exclusion criteria included: (1) patients with congenital aplastic anemia and (2) the presence of secondary aplastic anemia with definitive causes, such as severe liver, renal and heart function damage, severe uncontrolled infection, or any other uncontrolled illnesses.

2.2. Treatment protocol

Patients in the IST group received pATG (Wuhan Biotherapeutics, Co Lit, Wuhan, China) + CsA. In this group, an intravenous infusion of pATG (30 mg/kg/day) (from day 1 to day 5) was maintained for 18 hours a day for five consecutive days. The initial dose of CsA (from day eight until the end of the 2-year period) was 3–5 mg/kg/day and thereafter refined and adjusted to maintain a trough blood concentration of between 200–250 ng/mL. The duration of treatment for this group was 2 years.

Patients in the IST + E-PAG group received pATG + CsA + E-PAG (from day 8 until the end of the 6-month period) with the initial dose of 1 mg/kg/day (maximum dose 50 mg/day) that was subsequently adjusted according to the results of complete blood counts, and maintained for 6 months. For patients with hematologic responses, the dose of E-PAG was reduced by 50% every 2 months until the drug was eventually discontinued completely; whereas for those without hematologic responses, the dose was increased to a maximum of 50 mg/day to maintain the platelet counts $\geq 50 \times 10^9$/L. During the E-PAG dose adjustment phase, complete blood counts were performed at least once a week while liver and kidney function tests were performed at least once every 2 weeks. Once the dose was stabilized, and depending on the patient's condition, the testing interval was increased. For relapsed patients or those without hematologic response for 6 months, bone marrow biopsy, cytogenetic examination, and gene testing were performed again. Patients were followed up with clinic visits and phone calls.

2.3. Supportive therapies

In order to prevent serum sickness, methylprednisolone (2 mg/kg/day) with the dose tapered over one month, was administered. Platelet transfusions were provided to maintain a platelet count $\geq 50 \times 10^9$/L during the IST. Afterwards, platelets were transfused only for bleeding or a platelet count $<10 \times 10^9$/L. Packed red blood cell transfusions were provided to maintain hemoglobin levels $\geq$ 70 g/L. Granulocyte-colony-stimulating factor (G-CSF) (5 μg/kg/day) was provided to maintain neutrophils $>1 \times 10^9$/L to prevent from infection. Antifungal prophylaxis was offered routinely when ANC $<1 \times 10^9$/L. Neutropenic patients were treated in an isolation room. Blood cultures were performed for patients with neutropenia and fever. After performing the blood culture, broad-spectrum antibiotics would be administered and then adjusted accordingly when the culture results became available.

Patients who experienced relapses or did not respond to the treatment for 6 months received a second treatment of E-PAG, IST or an allogeneic hematopoietic stem cell transplantation.

2.4. Response evaluation

The primary endpoints were responses at 3 and 6 months, including complete responses (CRs) and partial responses (PRs). The secondary endpoints were the tolerability and toxicities of E-PAG, relapse, times to achieve transfusion independence, and overall survival (OS, calculated from the date of the IST starting to the day of death or the last date of follow-up).

The hematologic responses were defined as: CR, as an ANC at least 1.5×10^9/L, a hemoglobin level at least 110 g/L, and a platelet count at least 100×10^9/L; PR, as an ANC at least 0.5×10^9/L, a hemoglobin level at least 80 g/L, and a platelet count at least 20×10^9/L; and relapse was defined as peripheral blood counts declining to a level meeting the criteria for SAA or VSAA, after an initial attainment of CR or PR; Overall response rate (ORR) was defined as percentage of CR cases and PR cases in total cases; and complete response rate (CRR) was defined as percentage of CR cases in total cases.

Disease progression was defined as the development of the disease into clonal diseases such as paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS) and acute myeloid leukemia.

2.5. Statistical analysis

The statistical analyses were performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA). Measurement data that were not normally distributed were expressed as medians and ranges. The Kaplan–Meier method was used to analyze the overall survival (OS) and duration of the responses. Log-rank Fisher's exact tests were used to calculate the differences between treatment groups with a level of statistical significance of $p < 0.05$. The effect of response on survival and disease progression was analyzed by using the modeling response as a time-varying covariate in the Cox model. A univariate survival analysis was conducted using the Kaplan–Meier method, and survival was compared

Table 1. Baseline patient demongraphic characteristics in each treatment cohort.

Characteristics	IST + E-PAG (n = 18)	IST (n = 39)	*p*-values
Sex (male/female)	10/8	21/18	$p > 0.05$
Median age, y (range)	6.5(1.3-12.5)	7(1.6-13.5)	$p > 0.05$
White blood cell count, $\times 10^9$ /L	1.65(0.42-6.23)	1.86(0.38-6.54)	$p > 0.05$
Platelet count, $\times 10^9$ /L	6(1-18)	8(3-17)	$p > 0.05$
Hemoglobin, g/L	76(55-89)	78(53-92)	$p > 0.05$
Absolute neutrophil count, $\times 10^9$ /L	0.23(0.05-0.41)	0.28(0.06-0.45)	$p > 0.05$
Reticulocyte count, $\times 10^9$ /L	10.3(5.2-15.8)	9.2(4.6-17.1)	$p > 0.05$
Disease severity			$p > 0.05$
VSAA, n (%)	6(33.3)	14(35.8)	
SAA, n (%)	12(66.6)	25(64.1)	

Data are expressed as the number of cases (%) or median (range).
IST: Immunosupressive Therapy; E-PAG: Eltrombopag.

using the log-rank test. A multivariate survival analysis was performed using the Cox proportional hazard model.

3. Results

3.1. Patient characteristics

Baseline patient characteristics were similar between the two treatment cohorts (Table 1, $p > 0.05$). The median follow-up time for the IST group was 2076 (range, 1235–3105) days and that for the IST + E-PAG group was 926 (range, 568–1208) days.

3.2. Response rates

Significant differences were observed in the ORR and CRR at 6 months between the IST and IST + E-PAG groups (Table 2). At 3 months of treatment, the ORR in the IST group (n = 39) was 56.4% (95% CI 0.40–0.72), and the CRR was 12.8% (95% CI 0.18–0.24), which were not statistically lower than the ORR (77.7%, 95% CI 0.56–0.99, $p > 0.05$) and the CRR (22.2%, 95% CI 0.009–0.43, $p > 0.05$) in the IST + E-PAG group (n = 18), respectively. However, at 6 months of treatment, the ORR in the IST group was 69.2% (95% CI 0.54–0.84), and the CRR was 17.9% (95% CI 0.05–0.30), which were significantly lower than the ORR (94.4%, 95% CI 0.83–1.06, $p < 0.05$) and the CRR (50%, 95% CI 0.24–0.76, $p < 0.05$) in the IST + E-PAG group, respectively.

No significant differences were observed for ORR at 6 months between the two cohorts in terms of the absolute reticulocyte count (ARC) at diagnosis (75% for ARC $\leq$ 15 × 109/L vs 79.3% for ARC > 15 × 109/L $p > 0.05$), age (77.5% for age $\leq$ 12 years vs 76.4% for age > 12 years, $p > 0.05$), sex (80% for male vs 74% for female, $p > 0.05$), antibiotic treatment (78.9% for antibiotic treatment vs 73.6% for no antibiotic treatment, $p > 0.05$), G-CSF treatment in the IST group (71.4% for G-CSF treatment vs 66.6% for no G-CSF treatment, $p > 0.05$), G-CSF treatment in the IST + E-PAG group (100% for G-CSF treatment vs. 88.8% for no G-CSF treatment, $p > 0.05$) and disease severity (80% for SAA vs 75% for VSAA, $p > 0.05$).

The median time to achieve transfusion independence of red blood cells was 51 (range 0–523) days for the IST group and 35 (range 25–67) days for the IST + E-PAG group ($p < 0.05$), while the time to achieve transfusion independence of platelets was 53

Table 2. Response to immunosuppressive therapy vs. immunosuppressive therapy plus eltrombopag as first-line treatment in children with acquired aplastic anemia.

Time	3 mo			6 mo		
Response, n (%)	IST (n = 39)	IST + E-PAG (n = 18)	*p*-valus	IST (n = 39)	IST + E-PAG (n = 18)	*p*-values
ORR	22(56.4) (95% CI 0.40-0.72)	14 (77.7) (95% CI 0.56-0.99)	$p > 0.05$	27(69.2) (95% CI 0.54-0.84)	17(94.4) (95% CI 0.83-1.06)	$p < 0.05$
CR	5(12.8) (95% CI 0.18-0.24)	4 (22.2) (95% CI 0.009-0.43)	$p > 0.05$	7 (17.9) (95% CI 0.05-0.30)	9(50) (95% CI 0.24-0.76)	$p < 0.05$
PR	17 (43.5)	10 (55.5)		20 (51.2)	8(44.4)	
NR	17 (43.5)	4 (22.2)		12 (30.7)	0(0)	
Relapse	0(0)	0 (0)		0 (0)	1(5.5)	

CR: Complete response; PR: Partial response; NR, no response; ORR: CR + PR; IST: Immunosupressive Therapy; E-PAG: Eltrombopag.

(range 33–506) days for the IST group and 38 (range 27–92) days for the IST + E-PAG group ($p < 0.05$). Thirteen patients achieved transfusion independence after approximately 1 month of IST + E-PAG treatment. Furthermore, a significant increase in ANC at 8 weeks of IST + E-PAG treatment was observed with a median value of 0.38 (range 0.05–0.45) $\times$ 10^9/L before treatment vs. 1.36 (range 0.8–4.79) $\times$ 10^9/L after treatment ($p < 0.05$).

3.3. Further treatment

Twelve patients with no hematological responses were all from the IST group, with an ANC significantly lower than that of patients with a hematological response ($0.12 \pm 0.05 \times 10^9$/L vs. $0.28 \pm 0.07 \times 10^9$/L, $p < 0.05$). Seven of the 12 patients underwent allogeneic hematopoietic stem cell transplantation and six patients were still alive on the date of last follow up (Table 3). One patient died of severe immune rejection. Due to the lack of a suitable donor, three patients received a second IST course and were still alive on the date reported (Table 3). One patient died of an intracranial hemorrhage due to a low platelet count.

A patient with a PR died of a severe infection. A total of four patients relapsed, one from the IST + E-PAG group with the other three being from IST group. The patient from the IST + E-PAG group who relapsed at 6 months, possibly due to an irregular dosing of E-PAG, received a second IST course at eight months for a further 6 months (Table 3). After 2 months of treatment, the patient reached PR and was still alive on the date reported (Table 3). Among the other three patients who relapsed were from the IST group (one of whom relapsed at approximately 1 year and the other two at approximately 2 years), two of them underwent allogeneic hematopoietic stem cell transplantation are still currently alive (Table 3).

The 2-year overall survival (OS) rate was 92.3% in the IST group and 100% in the IST + E-PAG group ($p > 0.05$, Figure 1). The 2-year overall survival rate of patients with and without hematological response was 97.7% and 83.3%, respectively ($p > 0.05$, Figure 2). The 2-year overall survival rate of patients with CR, PR and NR was 100%, 96.4% and 83.3%, respectively ($p > 0.05$, Figure 3).

Table 3. Further treatment and response.

Treatment, n	IST (n = 39)	IST + E-PAG (n = 18)	Response at last follow-up
A second course of IST	3	0	2PR, 1NR
A second course of E-PAG	0	1	PR
Transplanted	9	0	2CR, 4PR, 3NR

IST: Immunosuppressive therapy; E-PAG: Eltrombopag; CR: Complete response; PR: Partial response; NR, no response.

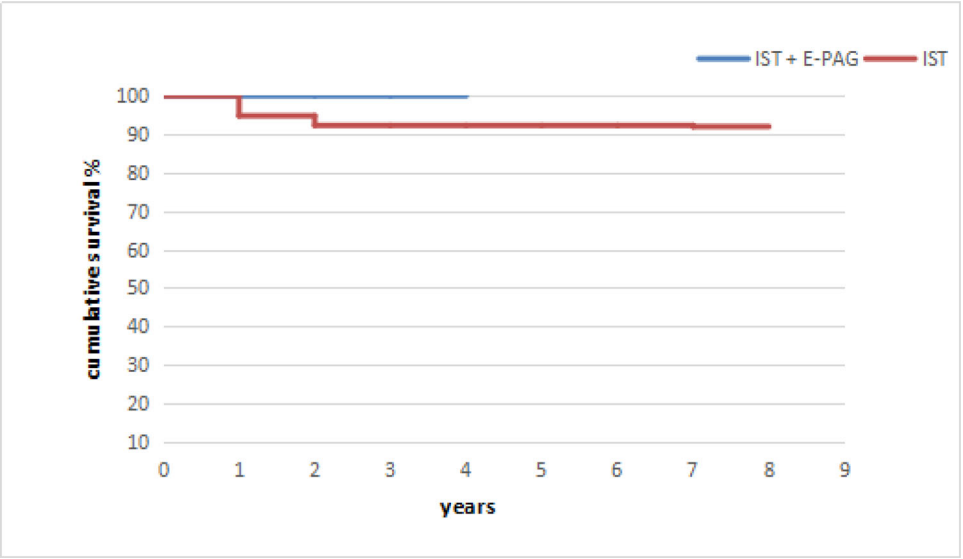

Figure 1. Overall survival (OS) of patients with aplastic anemia treated with immunosuppressive therapy (IST) alone compared to those treated with IST with eltrombopag (IST + E-PAG).

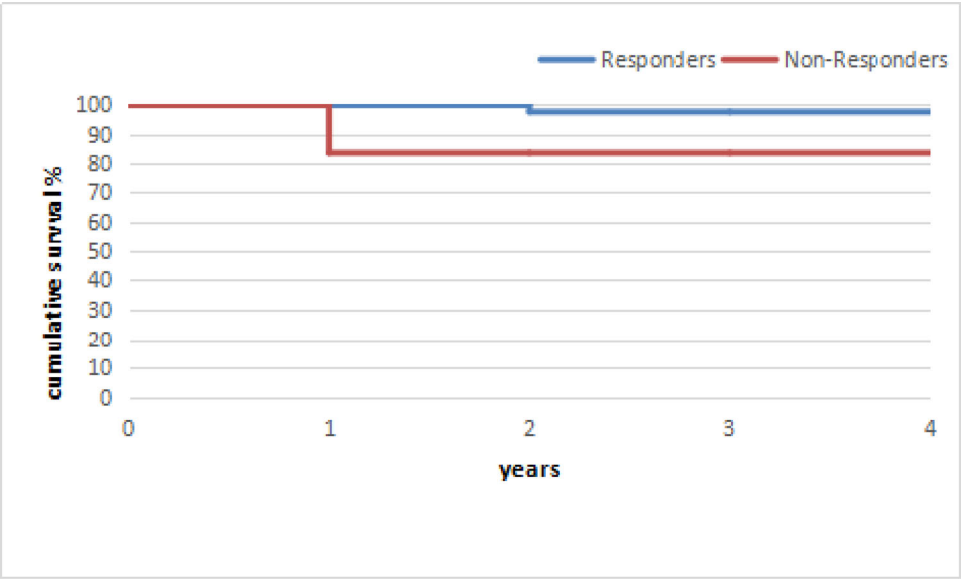

Figure 2. Overall survival (OS) of patients with aplastic anemia who achieved a clinical response in general in both treatment cohorts (complete response or partial response) compared with those who had no response.

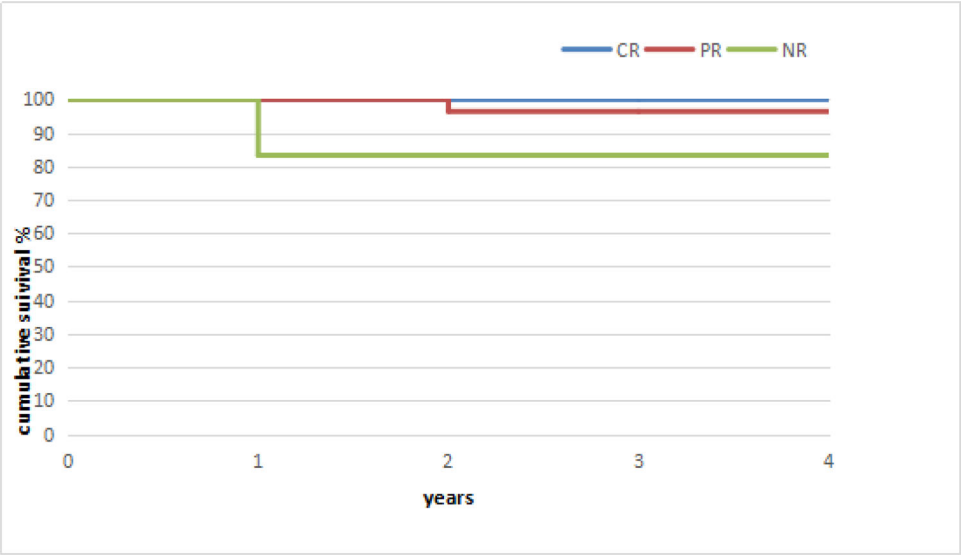

Figure 3. Overall survival (OS) of patients with aplastic anemia per response in both treatment cohorts. CR, complete response; NR, no response; PR, partial response.

The univariate and multivariate analyses for OS in terms of sex, age, disease severity, ARC at diagnosis, ANC at diagnosis, G-CSF treatment and prior antibiotic treatment showed that none of these factors significantly affected the OS.

3.4. Clonal evolution

There were no clinical manifestations that suggested clonal evolution.

3.5. Adverse reactions

During the ATG infusion and within 2–3 weeks after infusion, 36 patients developed fever and 15 developed serum sickness, all of which were controlled after active symptomatic treatment. The main side-effect of

E-PAG treatment was the slight to moderately elevated bilirubin levels which were temporary and controllable, accounting for about 66.6% (12/18) of patients in the IST + E-PAG group vs. 20.5% (8/39) of patients in the IST group ($p < 0.05$). Other adverse reactions were mild nausea, vomiting, and dizziness, all of which recovered spontaneously or improved after symptomatic treatment. None of the patients discontinued medication due to adverse reactions.

3.6. Economic analysis

The median first-time hospitalization expenses were RMB 82396 (range, 39323–154523, roughly $5774–22690 USD) yuan in the IST group and RMB54397 (range, 25624–134523, roughly $3762–19753 USD) yuan in the IST + E-PAG group ($p > 0.05$).

4. Discussion

E-PAG shows good tolerability and improves the response rates of adult patients with aplastic anemia.[21–26] In this retrospective study, we analyzed the safety and efficacy of E-PAG combined with IST in pediatric patients with SAA. A previous study showed an ORR of 74% and CR of 26% in children with SAA treated with IST.[13] I-Anne Huang et al also had a similar ORR.[28] While others have had better response rates,[16,17] which may be due to longer follow -up times, the inclusion of a transfusion-dependent None-SAA, and a stronger CsA tapering strategies for patients.[16,17] In this study, CSA was performed until the best response was obtained, then maintained for 3 months, followed by a slow tapering until discontinuation.[17] In our study, we found a little lower response rates in children with SAA treated with IST, which may be due to the smaller sample, higher proportion of female. Liu et al found that the response rate in the male group was better than the female group.[29] However, another study reported that an overall response rate was 53.5%, which may be due to the constrained resources, the inclusion of some forms of idiopathic bone marrow failure syndrome (IBMFS), or childhood MDS.[4]

When we added E-PAG to IST, the ORR increased to 94.4% and the CRR increased to 50%. These increases were statistically significant. Townsley et al. found similar results in patients with newly diagnosed SAA treated with the E-PAG + IST.[23] They observed that hematologic responses occurred in most patients at three months of treatment.[23] This is also consistent with our findings.

However, these results are not supported by Groarke et al, who found that E-PAG added to immunosuppression did not improve ORR at six months in pediatric patients with SAA.[27] We had found some important differences. First, in our study, the patients were significantly younger, with a median age of 6.5 years in the IST + EPAG group and seven years in the IST group, compared with the 15 years in the EPAG group and 11 years in the IST group in the study by Groarke et al. Age is an important predictor for the response to IST[30] and may influence the overall outcomes. Second, in our study the sample size was smaller. It will be important to observe whether this high ORR rate also occurs in studies on the addition of E-PAG in larger numbers of children. Third, selection bias and differences in supportive care measures may influence the difference in results. In our study, we used a second course of E-PAG in one relapsed patient, and then received a PR. Other studies have found similar results on aplastic anemia (AA) and idiopathic thrombocytopenic purpura.[21,31] Finally, ethnic differences may also have an effect on ORR. Studies have found CYP1A2 activity which is the hydrolase of E-PAG in Asians is significantly lower than in the Europeans.[32]

The median time periods to achieve transfusion independence in our study was approximately one month of E-PAG treatment, which was similar to the study by Townsley et al.[23] Furthermore, a significant difference in ANC at eight weeks after the treatment with E-PAG was observed. This may have been one of the factors for survival in the non-responders in our cohort, as the presence of an adequate neutrophil count would have prevented a severe febrile neutropenia.[33] The improvement in the health-related quality of life after E-PAG treatment that correlated with hematologic responses include: reduced risk of infection and bleeding, fewer blood transfusions, and fewer hospitalizations. All cause and SAA-related healthcare resource utilization (HCRU) were

shown to be reduced following E-PAG treatment.[34] In our study, the median first-time hospitalization expenses were lower in the IST + E-PAG group than that in the IST group, although there was no significant difference statistically.

One patient who relapsed from IST + E-PAG group at six months of treatment restarted E-PAG at eight months. After the treatment for additional two months, the patient reached PR again. This suggests that a new response can be expected on re-administration of the protocol after being discontinued. This is consistent with the findings from studies on aplastic anemia (AA) and idiopathic thrombocytopenic purpura.[21,31]

The mechanism of E-PAG action in bone marrow failure remains unclear. E-PAG activates only the JAK/STAT pathway in humans and chimpanzees and binds to the non-thrombopoietin binding site of C-MPL.[35] E-PAG does not compete with endogenous TPO, instead it has an enhancing effect. E-PAG can directly or indirectly regulate the immune abnormalities in patients with AA by promoting the secretion of regulator T cells, B cells, and transforming growth factor (TGF)-β, and the release of interferon-γ and tumor necrosis factor-α.[36,37] The mechanisms of action for E-PAG in improving bone marrow function in AA warrants further study.

The association between disease severity and response is controversial. Some studies have found that compared to children with SAA, children with VSAA have a superior CR and OS,[38] while others have found no significant differences between the two types of patients.[39] In our study, children with VSAA had a lower CR and OS than children with SAA, although there was no significant difference. This may be due to the greater mortality rate in VSAA patients and the presence of fewer residual hematopoietic stem cells in those patients. VSAA patients with ANC $< 0.2 \times 10^9$/L were more susceptible to infection, which is the leading cause of death in children with AA.[4]

Regarding the association between sex and response, we found no statistically significant difference between the two groups, although the response rate in the male group was better than the female group. This was consistent with the study by Liu et al.[29] Yoshida et al also found that young males experienced a faster recovery of bone marrow function following ATG.[40]

Tichelli et al found that the addition of G-CSF to standard ATG and CSA had no effect on OS, EFS, remission, relapse rates, and mortality.[41] In our study, we found that the addition of G-CSF to the treatment of both two groups did not show better results on the ORR. A meta-analysis about the use of growth factors in patients with aplastic anemia, including six randomized trials, did not show better results with the addition of hematopoietic growth factors on mortality, response rates, or infectious complications.[42] Some studies found that the ANC and ARC at diagnosis significantly affected the OS in the univariate analysis.[17] However, in our study, we found that neither the ARC and ANC at the time of diagnosis, nor the prior antibiotic treatment, significantly affected the OS. One of the explanations for this discrepancy may be due to the small sample size and the shorter follow-up time. Therefore, we consider that accumulation of more cases and a longer time follow up are warranted.

The clonal evolution of AA, the development of myelodysplastic syndromes and acute myelogenous leukemia were the key concerns of our extensive pretreatment and research on patients receiving additional E-PAG treatment.[21] In terms of cellular clonal

evolution, the use of E-PAG to date has not led to a significant increase in the cumulative incidence of cellular clonal genetic abnormalities compared to the historical experiences of AA treated with IST monotherapy.[18] In the present study, we followed up 57 patients for a median 2076 (range, 1235–3105) days in the IST group and for 926 (range, 568–1208) days in the IST + E-PAG group, and found that there were no clinical manifestations that suggested clonal evolution. However, due to the short-term follow-up, a definite conclusion on this aspect cannot be drawn, because the median time to clonal evolution of IST is generally considered to be four to six years; therefore, it is important to increase the follow-up durations of patients receiving E-PAG treatment in order to investigate this risk.

This study had several limitations. First, the sample size was relatively small. It will be important to observe whether this higher ORR rate also occurs in studies on the addition of E-PAG in larger numbers of children. Further research is needed to determine the initial recommended dose of E-PAG for the treatment of children with SAA. Second, the efficacy of treatment in different age groups and various degrees of disease severity was not assessed. Finally, the factors that may affect the therapeutic efficacy of E-PAG and the mechanism of clonal evolution induced by E-PAG were not determined. Further studies are needed to address these issues.

In summary, our findings suggest that E-PAG is safe and effective in children with SAA. It also has good reproducibility, good compliance, and controllable side-effects. The combination of E-PAG with IST may help patients to earn the time for allogenic HSCT or may allow the omittance of HSCT altogether.

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Disclosure statement

The authors declare no conflict of interest.

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ORCID

Sisi Li http://orcid.org/0000-0002-4862-8740

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, Yongmin Tang. The data are not publicly available due to ethical restrictions.

Contributors' statement

Meixin Fang conceptualized and designed the study, collected data, performed the data analysis and wrote the manuscript. Hua Song, Jingying Zhang, Sisi Li, Diying Shen: helped data analysis with constructive discussions. Yongmin Tang as the correspondence author takes the full responsibility of this manuscript with contribution for the conception of the study, data analysis, and manuscript amendment.

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·论著·

抗人T细胞猪免疫球蛋白治疗重型再生障碍性贫血患者的药物代谢动力学研究

井丽萍　张莉　周康　彭广新　李洋　樊慧慧　叶蕾　李园
李建平　宋琳　杨文睿　张凤奎
中国医学科学院血液病医院(中国医学科学院血液学研究所),实验血液学国家重点实验室,国家血液病临床医学研究中心,天津　300020
通信作者:张凤奎,Email:fkzhang@ihcams.ac.cn

【摘要】 目的　研究抗人T细胞猪免疫球蛋白(p-ATG)在重型再生障碍性贫血(SAA)患者的药物代谢特点。**方法**　2017年2月至2017年12月纳入接受p-ATG联合环孢素A(CsA)免疫抑制治疗的SAA患者,p-ATG剂量为20 mg·kg^{-1}·d^{-1},持续12 h静脉给药,连续5 d。应用三抗体夹心ELISA方法检测p-ATG血药浓度,药代动力学分析软件拟合,计算相关参数并绘制药物代谢曲线。随访记录不良事件并评估治疗后6个月血液学反应。**结果**　入组16例接受p-ATG治疗的SAA患者,女8例,男8例,中位年龄22(12~49)岁,中位体重62.5(37.5~82.0)kg。其中14例可进行p-ATG药代动力学评价。p-ATG在体内分布为二室模型,平均药物浓度峰值时间(T_{max})为(5.786±2.486)d,平均峰浓度(C_{max})为(616±452)mg/L,平均半衰期($T_{1/2}$)为(10.479±8.242)d。平均药物浓度时间曲线下面积[$AUC_{(0-t)}$]为(5.807±3.236)mg/L·d。14例患者治疗后6个月8例获得血液学反应,有效组与无效组患者$AUC_{(0-t)}$分别为(7.50±3.26)mg/L·d对(4.50±2.18)mg/L·d,C_{max}分别为(627±476)mg/L对(584±382)mg/L。**结论**　连续5 d输注后p-ATG血药浓度达峰值,后缓慢下降,半衰期10.479 d,用药后60 d体内检测到残存药物浓度。尚不能得出药物代谢与疗效及不良反应的关系。

【关键词】 贫血,再生障碍性;　抗人T细胞免疫球蛋白;　药代动力学
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Pharmacokinetic study of anti-human T-cell porcine immunoglobulin combined with cyclosporine A immunosuppressive therapy in patients with severe aplastic anemia

Jing Liping, Zhang Li, Zhou Kang, Peng Guangxin, Li Yang, Fan Huihui, Ye Lei, Li Yuan, Li Jianping, Song Lin, Yang Wenrui, Zhang Fengkui
State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking union Medical College, Tianjin 300020, China
Corresponding author: Zhang Fengkui, Email: fkzhang@ihcams.ac.cn

【Abstract】 Objective　To study the metabolic characteristics of anti-human T-cell porcine immunoglobulin (p-ATG) in patients with severe aplastic anemia (SAA). **Methods**　For patients with SAA treated with p-ATG combined cyclosporine A (CsA) immunosuppressants between February 2017 and December 2017, the p-ATG dose was 20 mg·kg^{-1}·d^{-1} over 12 h of intravenous administration for 5 consecutive days. The blood concentration of p-ATG was detected by the three-antibody sandwich ELISA method, the pharmacokinetic analysis software was fitted, and the second-chamber model method was used to calculate the pharmacokinetic parameters and plot the pharmacokinetic curve. Adverse events were recorded and the hematologic reactions were determined at 6 months after treatment. **Results**　Sixteen patients with SAA treated with p-ATG were enrolled, including 8 females and 8 males, with a median age of 22 years (range, 12 to 49 years) and a median weight of 62.5 kg (range, 37.5 to 82.0 kg). The pharmacokinetics of p-ATG could be evaluated in 14 cases. p-ATG is distributed in vivo as a two-chamber model, with an average drug concentration peak (T_{max}) of (5.786±2.486) days, a peak concentration (C_{max}) of (616±452) mg/L, and a half-life of (10.479±8.242) days. The area under the drug time curve (AUC)

was (5.807±3.236) mg/L·d. Six months after treatment, 8 of 14 patients received a hematologic response; the AUC (0-t) of the effective group and ineffective groups was (7.50±3.26) mg/L·d *vs* (4.50±2.18) mg/L·d, and the C_{max} was (627 ± 476) mg/L *vs* (584 ± 382) mg/L, respectively. **Conclusion** The plasma concentration of p-ATG reached a peak after 5 days of continuous infusion, and then decreased slowly, with a half-life of 10.479 days, and the residual drug concentration was detected in the body 60 days after administration. A relationship between drug metabolism and efficacy and adverse reactions could not be determined.

【Key words】 Anemia, aplastic; Antithymocyte globulin; Pharmacokinetics
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抗胸腺/淋巴细胞球蛋白(ATG/ALG)联合环孢素A(CsA)的免疫抑制治疗(IST)是不适合或者无法耐受造血干细胞移植(HSCT)的重型再生障碍性贫血(SAA)患者的首选治疗。抗人T细胞猪免疫球蛋白(p-ATG)是我国目前唯一自主生产的此类产品,临床应用该药物治疗SAA患者获得较好疗效,但目前国内外尚未见有关p-ATG治疗SAA的药代动力学报道。近期我们以接受p-ATG联合CsA治疗的SAA患者为研究对象,监测p-ATG的药物浓度变化,了解其药代动力学特性。

病例与方法

1. 方案设计:接受IST的SAA患者为中国医学科学院血液病医院住院患者,药物浓度检测单位为中国医药集团武汉生物制品研究所。依据赫尔辛基宣言原则及GCP规范,研究获得我院伦理委员会批准(批件号:ITT2014015-EC-2),试验前患者本人或其监护人均签署知情同意书。

2. 受试对象:纳入标准:2017年2月至2017年12月中国医学科学院血液病医院贫血诊疗中心住院,参照诊断标准[1]临床诊断为获得性SAA,按照《再生障碍性贫血诊断与治疗中国专家共识(2017年版)》[2]原则,因年龄或供者受限无法接受HSCT,拟接受IST的患者;ECOG评分≤2分;接受各项生化、心电图、超声、骨髓等诊断及筛查评估;患者本人或监护人签署知情同意书。排除标准:先天造血衰竭;患者合并无法控制的严重感染;合并肝肾心等重要脏器功能损伤;合并恶性肿瘤;妊娠或者哺乳期女性。随访至2018年12月,中位随访17(12~22)个月。

3. p-ATG给药方法与样品采集:p-ATG为中国武汉生物制品研究所产品,规格为每支250 mg。给药方案:20 mg·kg^{-1}·d^{-1}×5 d,皮试阴性后每天连续匀速静脉泵滴,持续12 h输注完毕。另一静脉通路按照1 mg/kg剂量泼尼松,换算为静脉短效氢化可的松(大约总量1/3),剩余剂量换算为静脉地塞米松输注,以预防即刻输注反应。开始p-ATG输注前采集静脉血为0 h,用药过程中观察记录患者的生命体征及药物的不良反应,如发生严重不良事件,立即暂停p-ATG输注,并采取急救措施。整个用药过程患者应用心电监护,并在医护人员的密切监护下层流病房内进行。

接受p-ATG治疗开始至停药后60 d连续不同时间点于用药过程中及用药后采集血样标本,时间点分别为第1天0、4、8、16 h,第2、3、4、5天(用药期间)静脉p-ATG输注前,第6、7、10、15、20、25、35、45、55、65天(停药后)晨起空腹采集静脉血标本。室温下静置30 min,24 ℃离心10 min,分离血清,置−70 ℃冰箱冷冻保存待测。

4. 血药浓度检测:采用三抗体夹心酶联免疫吸附(ELISA)方法,参照武汉生物制品研究所p-ATG检测试剂盒说明书进行血药浓度检测。应用兔抗猪IgG(美国Santa Cruz公司)包板,胎牛血清封闭;加入样品或对照100 μl,37 ℃温浴后洗板5次;然后加入HRP标记鼠抗猪IgG(美国Santa Cruz公司)100 μl,37 ℃温浴后洗板5次;再加入显色液反应15 min后,加入终止液终止反应;读取450 nm处吸光度(A)值,选择4参数方程式对阳性对照进行回归,计算获得待测样品浓度。实验重复2次取平均值。

5. 药代动力学模型的建立:采用DAS 3.1.4药代动力学分析软件进行拟合,确定权重和房室模型,计算药物代谢动力学参数,包括半衰期($T_{1/2}$),血药浓度-时间曲线下面积(AUC)、分布容积(V)和清除率(CL)等。AUC用梯形法计算,峰浓度(C_{max})、药物浓度达峰时间(T_{max})用实测值,并利用相关参数绘制药代曲线。

6. 疗效标准:参照中华医学会血液学分会制定的AA疗效标准[2],根据外周血常规恢复程度、是否依赖血制品输注等指标,将疗效分为完全缓解

（CR）、部分缓解（PR）、未缓解（NR），CR、PR共同计入血液学反应率。

7. 不良反应及各项临床指标观测：所有患者在IST期间均密切观察其临床症状体征，包括输注p-ATG期间的即刻不良事件及输注后3周内的血清病反应事件。并且在治疗期间监测淋巴细胞绝对值，观察其与药物浓度的相关性。治疗后监测血液骨髓相关指标，于治疗后6个月进行疗效评价，观察药代动力学指标与疗效的关系。

结　果

1. 入组患者一般情况：共纳入16例SAA患者，其中女8例，男8例，中位年龄22（12～49）岁，中位身高为162（130～170）cm，中位体重为62.5（37.5～82.0）kg，中位身体质量指数（BMI）为23.5（18.8～33.5）。其中SAA 13例，极重型再生障碍性贫血（VSAA）3例，中位外周血淋巴细胞绝对计数（ALC）为1.84（0.52～3.07）×10^9/L（表1）。其中2例患者因其他原因未能按方案完成p-ATG治疗，延长了用药时间，虽其药物浓度及代谢参数也进行了相应检验和安全性数据收集，在数据统计分析过程中仅保留安全性数据，其药代数据未予采用。

2. 基础结构模型的建立和药代模型参数：药物浓度时间曲线见图1。p-ATG输注第1天血药浓度均快速升高，在开始输注后的16 h最大血药浓度为（22.7±20.0）mg/L；p-ATG输注期间患者血药浓度仍逐渐升高，无明显平台期，并于输注结束后第1天达峰值血药浓度；结束末次药物输注后，p-ATG血药浓度开始下降，药物清除早期较快，后期缓慢，可体内存在长达60 d。

根据p-ATG在体内代谢浓度数值参数，采用DAS 3.1.4软件计算拟合，确定p-ATG药代动力学的二室模型（中央室及外周室），权重系数1/c/c。14例患者平均T_{max}为（5.786±2.486）d，平均C_{max}为（616±452）mg/L，平均$T_{1/2}$为（10.479±8.242）d，平均驻留时间［$MRT_{(0-\infty)}$］为（14.43±14.02）d，分布常数（0.581±0.432）1/d，分布速率（0.352±0.282）1/d，分布相半衰期（$T_{1/2\alpha}$）为（4.873±3.067）d，消除常数（6.100±5.439）1/d，消除速率（0.066±0.560）1/d，平均消除相半衰期（$T_{1/2\beta}$）（12.353±16.183）d，平均药物浓度时间曲线下面积［$AUC_{(0-t)}$］为（5.807±3.236）mg/L·d。

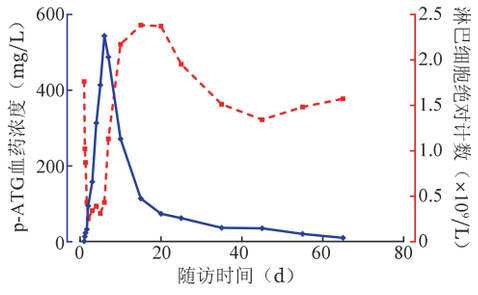
3. ALC、有效性与药物浓度相关性：14例患者中位基线ALC为1.84（0.52～3.07）×10^9/L，应用p-ATG过程中，ALC迅速下降，药物浓度达峰时患者中位ALC为0.31（0.10～0.90）×10^9/L，最低值仅为基线值的16.8%。随着ATG用药结束，药物浓度逐渐降低，ALC也逐渐复升，于用药14 d达峰值，中位水平为2.43（1.54～3.43）×10^9/L。此后，淋巴细胞再逐渐下降至接近基线水平，第35天中位ALC为1.71（0.72～2.27）×10^9/L（图1）。治疗后6个月，14例患

表1　16例入组抗人T细胞猪免疫球蛋白药代动力学检测SAA患者一般情况

例号	性别	年龄（岁）	诊断	体重（kg）	身高（cm）	BMI	治疗前血常规					
							ALC（×10^9/L）	WBC（×10^9/L）	HGB（g/L）	ANC（×10^9/L）	PLT（×10^9/L）	RET（%）
1	女	13	VSAA	46.0	154	19.4	3.07	3.2	66	0.13	14	6
2	男	21	SAA	61.0	170	21.1	1.90	2.6	47	0.70	15	12
3	女	12	SAA	44.0	153	18.8	1.93	2.4	69	0.47	11	12
4	男	38	SAA	80.0	170	27.7	1.96	2.2	70	0.24	11	6
5	女	13	SAA	74.0	162	28.2	1.69	2.5	50	0.41	2	72
6	女	48	SAA	82.5	157	33.5	1.26	2.0	57	0.74	9	15
7	男	31	SAA	62.5	166	22.7	2.09	2.5	66	0.41	10	23
8	男	22	SAA	72.5	170	25.1	0.52	0.97	70	0.45	9	20
9	男	12	VSAA	54.0	168	19.1	2.27	2.4	60	0.13	14	4
10	男	20	SAA	53.0	158	21.2	1.60	2.9	64	0.30	8	42
11	女	15	SAA	62.0	160	24.2	2.25	2.5	52	0.25	14	4
12	女	41	SAA	77.0	163	29.0	1.04	1.5	45	0.46	9	22
13	男	8	SAA	37.5	130	22.2	2.15	2.4	70	0.25	10	16
14	女	41	SAA	67.0	162	25.5	1.19	1.9	38	0.71	4	20
15	女	25	VSAA	56.0	166	20.3	0.72	0.8	58	0.03	18	2
16	男	11	SAA	65.0	156	26.7	1.77	2.0	78	0.23	13	11

注：SAA：重型再生障碍性贫血；VSAA：极重型再生障碍性贫血；ALC：淋巴细胞绝对计数；RET：网织红细胞比值

者中8例有效，有效组与无效组患者$AUC_{(0-t)}$分别为（7.50±3.26）mg/L·d、（4.50±2.18）mg/L·d，C_{max}分别为（627±476）mg/L、（584±382）mg/L，有效组均高于无效组，但样本量过低，未进行假设检验。



p-ATG：抗人T细胞猪免疫球蛋白

图1 14例重型再生障碍性贫血患者p-ATG血药浓度与淋巴细胞绝对计数动态曲线

4. p-ATG的耐受性：即刻输注不良反应主要为发热1例，皮疹1例，窦性心动过缓1例，加快糖皮质激素给药速度后患者症状缓解，未出现严重不良反应。药物治疗后7～14 d，2例患者出现血清病反应，1例为皮疹、发热，1例为水钠潴留，加大激素药物剂量均控制。

讨　论

ATG/ALG是提取人类胸腺细胞（包括T淋巴细胞及少许B淋巴细胞）及淋巴细胞（或淋巴细胞系）免疫动物（如兔、马、猪等），产生针对人T淋巴细胞的抗体，提纯后制备的含有IgG、IgM等多克隆免疫球蛋白，是一种针对人T细胞的强免疫抑制剂。目前有关AA的发病机制国际共识认为，T淋巴细胞的功能异常是导致骨髓造血衰竭的主要机制[3]，ATG/ALG是不能选择移植治疗的SAA患者的首选治疗方案，为避免患者过度免疫抑制而发生严重感染和复发，以及ATG/ALG药物血浓度过低而疗效欠佳，因此探索SAA患者个体化的最佳ATG/ALG剂量尤为重要。

p-ATG在中国AA患者体内药代动力学特点尚未明确，而应用p-ATG治疗AA的疗效及不良反应是否与其药代动力学相关，本研究希望为临床提供数据依据。采用三抗体夹心ELISA试验法，样本处理相对简单，成本费用较低，数据可靠等优点，具有广泛开展的可能性。本研究应用p-ATG总量为100 mg/kg，分5 d应用，每日输注时间不低于12 h，C_{max}为（616±452）mg/L，达到峰值为应用药物第6天，其后早期开始快速下降，第10天后开始缓慢下降，$T_{1/2}$为10.479 d，用药后60 d仍可检测到药物残留。

研究表明p-ATG为慢代谢药物，在总剂量相同的条件下，分多日应用，避免生物制剂的单次大剂量引起的短时药物浓度过高，从而引发高热、关节痛、过敏甚至休克等药物不良反应，且分日应用可维持更好的疗效，针对本病的异常T淋巴细胞抑制更充分持久。本研究14例患者平均$AUC_{(0-\infty)}$为（5.807±3.236）mg/L·d，患者随访至治疗后6个月时判断疗效，14例患者中8例有效，有效组$AUC_{(0-t)}$及C_{max}高于无效组，但样本量过低，未进行假设检验。本研究纳入患者样本量较小，视为Ⅰ期药代动力学研究，此结果没有足够效力评价疗效影响因素，加之AA疗效影响因素众多，故无法判定药代参数与疗效相关性。未来需增加样本量进一步研究。

由于ATG的消除半衰期很长，因此临床每日应用等量ATG剂量，体内p-ATG的浓度随应用时间逐步达稳态，这是很多药物产生临床治疗作用的先决条件[5]，同时也可能是药物产生不良反应的重要原因。本研究中所有患者未出现严重不良事件，因此根据p-ATG安全耐受的结果及药代动力学特点，我们认为在SAA治疗中，按100 mg/kg分5 d静脉输注是安全的。

本研究p-ATG T_{max}相较国内外报道马/猪ATG（h/p-ATG）相似[3-4,6]，其$T_{1/2\beta}$为12.353 d，短于r-ATG的21.56～29.67 d[4,6]，与既往p-ATG在HSCT患者中的10.99 d相似[7]。用药后60 d体内检测到残存药物浓度，短于r-ATG体内残留最长90 d的清除时间[4,6]，代谢清除相对较快。我们发现随着p-ATG体内的清除，患者淋巴细胞也很快恢复至较高水平，随着血药浓度的平稳，淋巴细胞也恢复至基线水平，这与文献报道r-ATG的IST后淋巴细胞较长时间抑制不同[8]，提示p-ATG免疫抑制强度可能略低，而与马-ATG（h-ATG）免疫抑制特征相近。

我们对SAA患者p-ATG药代特征研究表明，p-ATG是慢代谢的生物制剂，治疗过程随着药物的蓄积其浓度可快速达峰值，无明显平台期；治疗后药物清除早期较快，后期缓慢，可在体内长期残留。本研究尚不能得出药物代谢与疗效及不良反应的关系，尚需进一步研究探索。

利益冲突 所有作者声明无利益冲突

作者贡献声明 井丽萍：酝酿和设计实验、实施研究、采集数据、分析/解释数据、起草文章、统计分析；张凤奎：酝酿和设计实验、对文章的知识性内容作批评性审阅、指导；其他作者：实施研究、采集数据

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·读者·作者·编者·

2022年本刊可直接用英文缩写的常用词汇

血红蛋白　HGB
红细胞计数　RBC
白细胞计数　WBC
血小板计数　PLT
中性粒细胞绝对计数　ANC
丙氨酸转氨酶　ALT
天冬氨酸转氨酶　AST
谷氨酰转移酶　GGT
碱性磷酸酶　ALP
乳酸脱氢酶　LDH
凝血酶原时间　PT
部分激活的凝血活酶时间　APTT
红细胞生成素　EPO
血小板生成素　TPO
乙型肝炎病毒　HBV
丙型肝炎病毒　HCV
人类免疫缺陷病毒　HIV
核因子-κB　NF-κB
辅助性T淋巴细胞　Th细胞
调节性T淋巴细胞　Treg细胞
细胞毒性T淋巴细胞　CTL细胞
自然杀伤细胞　NK细胞
白细胞介素　IL
嵌合抗原受体T细胞　CAR-T细胞
肿瘤坏死因子　TNF
干细胞生长因子　SCF
粒细胞集落刺激因子　G-CSF
粒-巨噬细胞集落刺激因子　GM-CSF
巨噬细胞集落刺激因子　M-CSF
粒-巨噬细胞集落形成单位　CFU-GM
弥散性血管内凝血　DIC
实时荧光定量PCR　RQ-PCR
磁共振成像　MRI
正电子发射断层扫描　PET
荧光原位杂交　FISH
(1,3)-β-D葡聚糖检测　G试验
半乳甘露聚糖检测　GM试验
酶联免疫吸附实验　ELISA
噻唑蓝实验　MTT实验
磷酸盐缓冲液　PBS
胎牛血清　FBS
乙二胺四乙酸　EDTA
二甲基亚砜　DMSO
十二烷基硫酸钠-聚丙烯酰胺凝胶电泳　SDS-PAGE
美国国家综合癌症网络　NCCN
国际预后积分系统　IPSS
国际预后指数　IPI
异基因造血干细胞移植　allo-HSCT
自体造血干细胞移植　auto-HSCT
移植物抗宿主病　GVHD
人类白细胞抗原　HLA
受试者工作特征曲线　ROC曲线
常见不良反应事件评价标准　CTCAE

本刊编辑部

·论著·

抗人T细胞猪免疫球蛋白治疗重型再生障碍性贫血患者的药物代谢动力学研究

井丽萍　张莉　周康　彭广新　李洋　樊慧慧　叶蕾　李园
李建平　宋琳　杨文睿　张凤奎
中国医学科学院血液病医院(中国医学科学院血液学研究所),实验血液学国家重点实验室,国家血液病临床医学研究中心,天津　300020
通信作者:张凤奎,Email:fkzhang@ihcams.ac.cn

【摘要】 目的　研究抗人T细胞猪免疫球蛋白(p-ATG)在重型再生障碍性贫血(SAA)患者的药物代谢特点。**方法**　2017年2月至2017年12月纳入接受p-ATG联合环孢素A(CsA)免疫抑制治疗的SAA患者,p-ATG剂量为20 mg·kg^{-1}·d^{-1},持续12 h静脉给药,连续5 d。应用三抗体夹心ELISA方法检测p-ATG血药浓度,药代动力学分析软件拟合,计算相关参数并绘制药物代谢曲线。随访记录不良事件并评估治疗后6个月血液学反应。**结果**　入组16例接受p-ATG治疗的SAA患者,女8例,男8例,中位年龄22(12~49)岁,中位体重62.5(37.5~82.0)kg。其中14例可进行p-ATG药代动力学评价。p-ATG在体内分布为二室模型,平均药物浓度峰值时间(T_{max})为(5.786±2.486)d,平均峰浓度(C_{max})为(616±452)mg/L,平均半衰期($T_{1/2}$)为(10.479±8.242)d。平均药物浓度时间曲线下面积[$AUC_{(0-t)}$]为(5.807±3.236)mg/L·d。14例患者治疗后6个月8例获得血液学反应,有效组与无效组患者$AUC_{(0-t)}$分别为(7.50±3.26)mg/L·d对(4.50±2.18)mg/L·d,C_{max}分别为(627±476)mg/L对(584±382)mg/L。**结论**　连续5 d输注后p-ATG血药浓度达峰值,后缓慢下降,半衰期10.479 d,用药后60 d体内检测到残存药物浓度。尚不能得出药物代谢与疗效及不良反应的关系。

【关键词】 贫血,再生障碍性;　抗人T细胞免疫球蛋白;　药代动力学
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Pharmacokinetic study of anti-human T-cell porcine immunoglobulin combined with cyclosporine A immunosuppressive therapy in patients with severe aplastic anemia

Jing Liping, Zhang Li, Zhou Kang, Peng Guangxin, Li Yang, Fan Huihui, Ye Lei, Li Yuan, Li Jianping, Song Lin, Yang Wenrui, Zhang Fengkui
State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking union Medical College, Tianjin 300020, China
Corresponding author: Zhang Fengkui, Email: fkzhang@ihcams.ac.cn

【Abstract】 Objective　To study the metabolic characteristics of anti-human T-cell porcine immunoglobulin (p-ATG) in patients with severe aplastic anemia (SAA). **Methods**　For patients with SAA treated with p-ATG combined cyclosporine A (CsA) immunosuppressants between February 2017 and December 2017, the p-ATG dose was 20 mg·kg^{-1}·d^{-1} over 12 h of intravenous administration for 5 consecutive days. The blood concentration of p-ATG was detected by the three-antibody sandwich ELISA method, the pharmacokinetic analysis software was fitted, and the second-chamber model method was used to calculate the pharmacokinetic parameters and plot the pharmacokinetic curve. Adverse events were recorded and the hematologic reactions were determined at 6 months after treatment. **Results**　Sixteen patients with SAA treated with p-ATG were enrolled, including 8 females and 8 males, with a median age of 22 years (range, 12 to 49 years) and a median weight of 62.5 kg (range, 37.5 to 82.0 kg). The pharmacokinetics of p-ATG could be evaluated in 14 cases. p-ATG is distributed in vivo as a two-chamber model, with an average drug concentration peak (T_{max}) of (5.786±2.486) days, a peak concentration (C_{max}) of (616±452) mg/L, and a half-life of (10.479±8.242) days. The area under the drug time curve (AUC)

was (5.807±3.236) mg/L·d. Six months after treatment, 8 of 14 patients received a hematologic response; the AUC (0-t) of the effective group and ineffective groups was (7.50±3.26) mg/L·d *vs* (4.50±2.18) mg/L·d, and the C_{max} was (627 ± 476) mg/L *vs* (584 ± 382) mg/L, respectively. **Conclusion** The plasma concentration of p-ATG reached a peak after 5 days of continuous infusion, and then decreased slowly, with a half-life of 10.479 days, and the residual drug concentration was detected in the body 60 days after administration. A relationship between drug metabolism and efficacy and adverse reactions could not be determined.

【**Key words**】 Anemia, aplastic; Antithymocyte globulin; Pharmacokinetics
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抗胸腺/淋巴细胞球蛋白(ATG/ALG)联合环孢素A(CsA)的免疫抑制治疗(IST)是不适合或者无法耐受造血干细胞移植(HSCT)的重型再生障碍性贫血(SAA)患者的首选治疗。抗人T细胞猪免疫球蛋白(p-ATG)是我国目前唯一自主生产的此类产品,临床应用该药物治疗SAA患者获得较好疗效,但目前国内外尚未见有关p-ATG治疗SAA的药代动力学报道。近期我们以接受p-ATG联合CsA治疗的SAA患者为研究对象,监测p-ATG的药物浓度变化,了解其药代动力学特性。

病例与方法

1. 方案设计:接受IST的SAA患者为中国医学科学院血液病医院住院患者,药物浓度检测单位为中国医药集团武汉生物制品研究所。依据赫尔辛基宣言原则及GCP规范,研究获得我院伦理委员会批准(批件号:ITT2014015-EC-2),试验前患者本人或其监护人均签署知情同意书。

2. 受试对象:纳入标准:2017年2月至2017年12月中国医学科学院血液病医院贫血诊疗中心住院,参照诊断标准[1]临床诊断为获得性SAA,按照《再生障碍性贫血诊断与治疗中国专家共识(2017年版)》[2]原则,因年龄或供者受限无法接受HSCT,拟接受IST的患者;ECOG评分≤2分;接受各项生化、心电图、超声、骨髓等诊断及筛查评估;患者本人或监护人签署知情同意书。排除标准:先天造血衰竭;患者合并无法控制的严重感染;合并肝肾心等重要脏器功能损伤;合并恶性肿瘤;妊娠或者哺乳期女性。随访至2018年12月,中位随访17(12~22)个月。

3. p-ATG给药方法与样品采集:p-ATG为中国武汉生物制品研究所产品,规格为每支250 mg。给药方案:20 mg·kg^{-1}·d^{-1}×5 d,皮试阴性后每天连续匀速静脉泵滴,持续12 h输注完毕。另一静脉通路按照1 mg/kg剂量泼尼松,换算为静脉短效氢化可的松(大约总量1/3),剩余剂量换算为静脉地塞米松输注,以预防即刻输注反应。开始p-ATG输注前采集静脉血为0 h,用药过程中观察记录患者的生命体征及药物的不良反应,如发生严重不良事件,立即暂停p-ATG输注,并采取急救措施。整个用药过程患者应用心电监护,并在医护人员的密切监护下层流病房内进行。

接受p-ATG治疗开始至停药后60 d连续不同时间点于用药过程中及用药后采集血样标本,时间点分别为第1天0、4、8、16 h,第2、3、4、5天(用药期间)静脉p-ATG输注前,第6、7、10、15、20、25、35、45、55、65天(停药后)晨起空腹采集静脉血标本。室温下静置30 min,24 ℃离心10 min,分离血清,置−70 ℃冰箱冷冻保存待测。

4. 血药浓度检测:采用三抗体夹心酶联免疫吸附(ELISA)方法,参照武汉生物制品研究所p-ATG检测试剂盒说明书进行血药浓度检测。应用兔抗猪IgG(美国Santa Cruz公司)包板,胎牛血清封闭;加入样品或对照100 μl,37 ℃温浴后洗板5次;然后加入HRP标记鼠抗猪IgG(美国Santa Cruz公司)100 μl,37 ℃温浴后洗板5次;再加入显色液反应15 min后,加入终止液终止反应;读取450 nm处吸光度(A)值,选择4参数方程式对阳性对照进行回归,计算获得待测样品浓度。实验重复2次取平均值。

5. 药代动力学模型的建立:采用DAS 3.1.4药代动力学分析软件进行拟合,确定权重和房室模型,计算药物代谢动力学参数,包括半衰期($T_{1/2}$),血药浓度-时间曲线下面积(AUC)、分布容积(V)和清除率(CL)等。AUC用梯形法计算,峰浓度(C_{max})、药物浓度达峰时间(T_{max})用实测值,并利用相关参数绘制药代曲线。

6. 疗效标准:参照中华医学会血液学分会制定的AA疗效标准[2],根据外周血常规恢复程度、是否依赖血制品输注等指标,将疗效分为完全缓解

（CR）、部分缓解（PR）、未缓解（NR），CR、PR共同计入血液学反应率。

7. 不良反应及各项临床指标观测：所有患者在IST期间均密切观察其临床症状体征，包括输注p-ATG期间的即刻不良事件及输注后3周内的血清病反应事件。并且在治疗期间监测淋巴细胞绝对值，观察其与药物浓度的相关性。治疗后监测血液骨髓相关指标，于治疗后6个月进行疗效评价，观察药代动力学指标与疗效的关系。

结　果

1. 入组患者一般情况：共纳入16例SAA患者，其中女8例，男8例，中位年龄22（12～49）岁，中位身高为162（130～170）cm，中位体重为62.5（37.5～82.0）kg，中位身体质量指数（BMI）为23.5（18.8～33.5）。其中SAA 13例，极重型再生障碍性贫血（VSAA）3例，中位外周血淋巴细胞绝对计数（ALC）为1.84（0.52～3.07）×10^9/L（表1）。其中2例患者因其他原因未能按方案完成p-ATG治疗，延长了用药时间，虽其药物浓度及代谢参数也进行了相应检验和安全性数据收集，在数据统计分析过程中仅保留安全性数据，其药代数据未予采用。

2. 基础结构模型的建立和药代模型参数：药物浓度时间曲线见图1。p-ATG输注第1天血药浓度均快速升高，在开始输注后的16 h最大血药浓度为（22.7±20.0）mg/L；p-ATG输注期间患者血药浓度仍逐渐升高，无明显平台期，并于输注结束后第1天达峰值血药浓度；结束末次药物输注后，p-ATG血药浓度开始下降，药物清除早期较快，后期缓慢，可体内存在长达60 d。

根据p-ATG在体内代谢浓度数值参数，采用DAS 3.1.4软件计算拟合，确定p-ATG药代动力学的二室模型（中央室及外周室），权重系数1/c/c。14例患者平均T_{max}为（5.786±2.486）d，平均C_{max}为（616±452）mg/L，平均$T_{1/2}$为（10.479±8.242）d，平均驻留时间［$MRT_{(0-\infty)}$］为（14.43±14.02）d，分布常数（0.581±0.432）1/d，分布速率（0.352±0.282）1/d，分布相半衰期（$T_{1/2\alpha}$）为（4.873±3.067）d，消除常数（6.100±5.439）1/d，消除速率（0.066±0.560）1/d，平均消除相半衰期（$T_{1/2\beta}$）（12.353±16.183）d，平均药物浓度时间曲线下面积［$AUC_{(0-t)}$］为（5.807±3.236）mg/L·d。

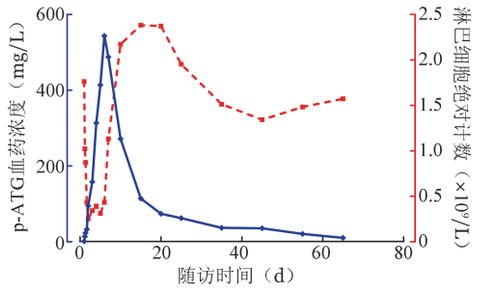
3. ALC、有效性与药物浓度相关性：14例患者中位基线ALC为1.84（0.52～3.07）×10^9/L，应用p-ATG过程中，ALC迅速下降，药物浓度达峰时患者中位ALC为0.31（0.10～0.90）×10^9/L，最低值仅为基线值的16.8%。随着ATG用药结束，药物浓度逐渐降低，ALC也逐渐复升，于用药14 d达峰值，中位水平为2.43（1.54～3.43）×10^9/L。此后，淋巴细胞再逐渐下降至接近基线水平，第35天中位ALC为1.71（0.72～2.27）×10^9/L（图1）。治疗后6个月，14例患

表1　16例入组抗人T细胞猪免疫球蛋白药代动力学检测SAA患者一般情况

例号	性别	年龄（岁）	诊断	体重（kg）	身高（cm）	BMI	治疗前血常规					
							ALC（×10^9/L）	WBC（×10^9/L）	HGB（g/L）	ANC（×10^9/L）	PLT（×10^9/L）	RET（%）
1	女	13	VSAA	46.0	154	19.4	3.07	3.2	66	0.13	14	6
2	男	21	SAA	61.0	170	21.1	1.90	2.6	47	0.70	15	12
3	女	12	SAA	44.0	153	18.8	1.93	2.4	69	0.47	11	12
4	男	38	SAA	80.0	170	27.7	1.96	2.2	70	0.24	11	6
5	女	13	SAA	74.0	162	28.2	1.69	2.5	50	0.41	2	72
6	女	48	SAA	82.5	157	33.5	1.26	2.0	57	0.74	9	15
7	男	31	SAA	62.5	166	22.7	2.09	2.5	66	0.41	10	23
8	男	22	SAA	72.5	170	25.1	0.52	0.97	70	0.45	9	20
9	男	12	VSAA	54.0	168	19.1	2.27	2.4	60	0.13	14	4
10	男	20	SAA	53.0	158	21.2	1.60	2.9	64	0.30	8	42
11	女	15	SAA	62.0	160	24.2	2.25	2.5	52	0.25	14	4
12	女	41	SAA	77.0	163	29.0	1.04	1.5	45	0.46	9	22
13	男	8	SAA	37.5	130	22.2	2.15	2.4	70	0.25	10	16
14	女	41	SAA	67.0	162	25.5	1.19	1.9	38	0.71	4	20
15	女	25	VSAA	56.0	166	20.3	0.72	0.8	58	0.03	18	2
16	男	11	SAA	65.0	156	26.7	1.77	2.0	78	0.23	13	11

注：SAA：重型再生障碍性贫血；VSAA：极重型再生障碍性贫血；ALC：淋巴细胞绝对计数；RET：网织红细胞比值

者中8例有效，有效组与无效组患者$AUC_{(0-t)}$分别为（7.50±3.26）mg/L·d、（4.50±2.18）mg/L·d，C_{max}分别为（627±476）mg/L、（584±382）mg/L，有效组均高于无效组，但样本量过低，未进行假设检验。



p-ATG：抗人T细胞猪免疫球蛋白

图1 14例重型再生障碍性贫血患者p-ATG血药浓度与淋巴细胞绝对计数动态曲线

4. p-ATG的耐受性：即刻输注不良反应主要为发热1例，皮疹1例，窦性心动过缓1例，加快糖皮质激素给药速度后患者症状缓解，未出现严重不良反应。药物治疗后7～14 d，2例患者出现血清病反应，1例为皮疹、发热，1例为水钠潴留，加大激素药物剂量均控制。

讨　论

ATG/ALG是提取人类胸腺细胞（包括T淋巴细胞及少许B淋巴细胞）及淋巴细胞（或淋巴细胞系）免疫动物（如兔、马、猪等），产生针对人T淋巴细胞的抗体，提纯后制备的含有IgG、IgM等多克隆免疫球蛋白，是一种针对人T细胞的强免疫抑制剂。目前有关AA的发病机制国际共识认为，T淋巴细胞的功能异常是导致骨髓造血衰竭的主要机制[3]，ATG/ALG是不能选择移植治疗的SAA患者的首选治疗方案，为避免患者过度免疫抑制而发生严重感染和复发，以及ATG/ALG药物血浓度过低而疗效欠佳，因此探索SAA患者个体化的最佳ATG/ALG剂量尤为重要。

p-ATG在中国AA患者体内药代动力学特点尚未明确，而应用p-ATG治疗AA的疗效及不良反应是否与其药代动力学相关，本研究希望为临床提供数据依据。采用三抗体夹心ELISA试验法，样本处理相对简单，成本费用较低，数据可靠等优点，具有广泛开展的可能性。本研究应用p-ATG总量为100 mg/kg，分5 d应用，每日输注时间不低于12 h，C_{max}为（616±452）mg/L，达到峰值为应用药物第6天，其后早期开始快速下降，第10天后开始缓慢下降，$T_{1/2}$为10.479 d，用药后60 d仍可检测到药物残留。

研究表明p-ATG为慢代谢药物，在总剂量相同的条件下，分多日应用，避免生物制剂的单次大剂量引起的短时药物浓度过高，从而引发高热、关节痛、过敏甚至休克等药物不良反应，且分日应用可维持更好的疗效，针对本病的异常T淋巴细胞抑制更充分持久。本研究14例患者平均$AUC_{(0-\infty)}$为（5.807±3.236）mg/L·d，患者随访至治疗后6个月时判断疗效，14例患者中8例有效，有效组$AUC_{(0-t)}$及C_{max}高于无效组，但样本量过低，未进行假设检验。本研究纳入患者样本量较小，视为Ⅰ期药代动力学研究，此结果没有足够效力评价疗效影响因素，加之AA疗效影响因素众多，故无法判定药代参数与疗效相关性。未来需增加样本量进一步研究。

由于ATG的消除半衰期很长，因此临床每日应用等量ATG剂量，体内p-ATG的浓度随应用时间逐步达稳态，这是很多药物产生临床治疗作用的先决条件[5]，同时也可能是药物产生不良反应的重要原因。本研究中所有患者未出现严重不良事件，因此根据p-ATG安全耐受的结果及药代动力学特点，我们认为在SAA治疗中，按100 mg/kg分5 d静脉输注是安全的。

本研究p-ATG T_{max}相较国内外报道马/猪ATG（h/p-ATG）相似[3-4,6]，其$T_{1/2\beta}$为12.353 d，短于r-ATG的21.56～29.67 d[4,6]，与既往p-ATG在HSCT患者中的10.99 d相似[7]。用药后60 d体内检测到残存药物浓度，短于r-ATG体内残留最长90 d的清除时间[4,6]，代谢清除相对较快。我们发现随着p-ATG体内的清除，患者淋巴细胞也很快恢复至较高水平，随着血药浓度的平稳，淋巴细胞也恢复至基线水平，这与文献报道r-ATG的IST后淋巴细胞较长时间抑制不同[8]，提示p-ATG免疫抑制强度可能略低，而与马-ATG（h-ATG）免疫抑制特征相近。

我们对SAA患者p-ATG药代特征研究表明，p-ATG是慢代谢的生物制剂，治疗过程随着药物的蓄积其浓度可快速达峰值，无明显平台期；治疗后药物清除早期较快，后期缓慢，可在体内长期残留。本研究尚不能得出药物代谢与疗效及不良反应的关系，尚需进一步研究探索。

利益冲突 所有作者声明无利益冲突

作者贡献声明 井丽萍：酝酿和设计实验、实施研究、采集数据、分析/解释数据、起草文章、统计分析；张凤奎：酝酿和设计实验、对文章的知识性内容作批评性审阅、指导；其他作者：实施研究、采集数据

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·读者·作者·编者·

2022年本刊可直接用英文缩写的常用词汇

血红蛋白　HGB
红细胞计数　RBC
白细胞计数　WBC
血小板计数　PLT
中性粒细胞绝对计数　ANC
丙氨酸转氨酶　ALT
天冬氨酸转氨酶　AST
谷氨酰转移酶　GGT
碱性磷酸酶　ALP
乳酸脱氢酶　LDH
凝血酶原时间　PT
部分激活的凝血活酶时间　APTT
红细胞生成素　EPO
血小板生成素　TPO
乙型肝炎病毒　HBV
丙型肝炎病毒　HCV
人类免疫缺陷病毒　HIV
核因子-κB　NF-κB
辅助性T淋巴细胞　Th细胞
调节性T淋巴细胞　Treg细胞
细胞毒性T淋巴细胞　CTL细胞
自然杀伤细胞　NK细胞
白细胞介素　IL
嵌合抗原受体T细胞　CAR-T细胞
肿瘤坏死因子　TNF
干细胞生长因子　SCF
粒细胞集落刺激因子　G-CSF
粒-巨噬细胞集落刺激因子　GM-CSF
巨噬细胞集落刺激因子　M-CSF
粒-巨噬细胞集落形成单位　CFU-GM
弥散性血管内凝血　DIC
实时荧光定量PCR　RQ-PCR
磁共振成像　MRI
正电子发射断层扫描　PET
荧光原位杂交　FISH
(1,3)-β-D葡聚糖检测　G试验
半乳甘露聚糖检测　GM试验
酶联免疫吸附实验　ELISA
噻唑蓝实验　MTT实验
磷酸盐缓冲液　PBS
胎牛血清　FBS
乙二胺四乙酸　EDTA
二甲基亚砜　DMSO
十二烷基硫酸钠-聚丙烯酰胺凝胶电泳　SDS-PAGE
美国国家综合癌症网络　NCCN
国际预后积分系统　IPSS
国际预后指数　IPI
异基因造血干细胞移植　allo-HSCT
自体造血干细胞移植　auto-HSCT
移植物抗宿主病　GVHD
人类白细胞抗原　HLA
受试者工作特征曲线　ROC曲线
常见不良反应事件评价标准　CTCAE

本刊编辑部

THERAPEUTIC ADVANCES in
Hematology

Original Research

Antihuman T lymphocyte porcine immunoglobulin combined with cyclosporine as first-line immunosuppressive therapy for severe aplastic anemia in China: a large single-center, 10-year retrospective study

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Wenrui Yang, Xu Liu, Xin Zhao, Li Zhang, Guangxin Peng, Lei Ye, Kang Zhou, Yuan Li, Jianping Li, Huihui Fan, Yang Yang, Youzhen Xiong, Liping Jing and Fengkui Zhang

Abstract

Background: Antihuman T lymphocyte porcine immunoglobulin (p-ATG) has been the most common ATG preparation in immunosuppressive therapy (IST) in Chinese patients with severe aplastic anemia (SAA) since 2009.

Objectives: This study aimed to evaluate the early hematologic response and long-term outcomes of a large cohort of patients with SAA who received p-ATG plus cyclosporine (CsA) as first-line therapy from 2010 to 2019.

Design: This is a single-center retrospective study of medical records.

Methods: We analyzed the data of 1023 consecutive patients with acquired aplastic anemia (AA) who underwent p-ATG combined with CsA as a first-line IST treatment from 2010 to 2019 at our department.

Results: The median age of the patients was 24 (4–75) years, and the median follow-up time was 57.2 months (3 days–137.5 months). There was an early mortality rate of 2.8% with a median death time of 0.9 months (3 days–2.9 months). The overall response rates were 40.6% and 56.1% at 3 and 6 months, respectively. The 5-year cumulative incidences of relapse and clonal evolution were 9.0% [95% confidence interval (CI) = 4.2–16.0%] and 4.5% (95% CI = 1.4–10.6%), respectively. The 5-year overall survival (OS) and event-free survival rates were 83.7% (95% CI = 81.1–86.0%) and 50.4% (95% CI = 47.1–53.5%), respectively.

Conclusion: p-ATG combined with CsA for the treatment of AA is effective and safe, and p-ATG can be used as an alternative ATG preparation for the standard IST regimen in areas in which h-ATG is not available.

Keywords: aplastic anemia, hematologic response, IST, p-ATG, survival

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Correspondence to:
Liping Jing
State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China Tianjin Institutes of Health Science, Tianjin 301600, China.
jingliping@ihcams.ac.cn

Wenrui Yang
Xu Liu
Xin Zhao
Li Zhang
Guangxin Peng
Lei Ye
Kang Zhou
Yuan Li
Jianping Li
Huihui Fan
Yang Yang
Youzhen Xiong
Fengkui Zhang
State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

Wenrui Yang, Xu Liu, Xin Zhao, Li Zhang, Guangxin Peng, Lei Ye, Kang Zhou, Yuan Li, Jianping Li, Huihui Fan, Yang Yang, Youzhen Xiong, Fengkui Zhang is also affiliated to Tianjin Institutes of Health Science, Tianjin, China

Introduction

The first report of the beneficial effect of antilymphocyte globulin (ALG) on autologous hematologic recovery in severe aplastic anemia (SAA) was done by Methe *et al.*[1] in 1970. In a series of subsequent studies, antihuman thymocyte globulin (ATG) combined with cyclosporine (CsA), as a standard intensive immunosuppressive regimen, was found to be a well-established first-line treatment for patients with SAA who are ineligible for hematopoietic stem cell transplantation.[2–9]

ATG/ALG is obtained from the immunization of animals, generally horses or rabbits, with human lymphoid cells, such as thymus (ATG) and thoracic duct lymphocytes (ALG).[10] Previous prospective

and retrospective studies demonstrated that horse antihuman thymocyte globulin (h-ATG) was superior to rabbit ATG in terms of both hematologic response (HR) and survival.[11–15] Therefore, h-ATG is recommended as the standard preparation for first-line immune suppression in SAA. Nevertheless, rabbit antihuman thymocyte globulin (r-ATG) combined with CsA is still widely used as a first-line immunosuppressive regimen in Europe and Asia because h-ATG (lymphoglobulin) was withdrawn from the market in 2007 and replaced by r-ATG, which is the only available formulation of ATG.

As in Europe, h-ATG has been unavailable in China since the year 2000. The antihuman T lymphocyte porcine immunoglobulin (p-ATG, provided by Yujin Bio-Pharma Wuhan CNBG Co., Ltd., Wuhan, China) was made available in 1983 and approved for the treatment of aplastic anemia by the Sino Food and Drug Administration in 2004. It is a commonly used ATG preparation in immunosuppressive therapy (IST) for SAA in China, even exceeding the r-ATG usage since 2009. A few small retrospective cohort studies reported that p-ATG combined with cyclosporine (CsA) is a useful immunosuppressive regimen for SAA in China.[16–19] This study aimed to evaluate the early HR and long-term outcomes of a large cohort of patients with SAA who received p-ATG plus CsA as first-line therapy from 2010 to 2019.

Methods

Patients

This study retrospectively analyzed the data of 1023 consecutive patients diagnosed with SAA admitted to the Anemia Therapeutic Center of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, between January 2010 and December 2019. The study protocol was approved by the institutional ethics committee (IIT2021008-EC-1) and follows the Declaration of Helsinki. The reporting of this study conforms to STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines.[20]

All 1023 patients with a confirmed diagnosis of transfusion-dependent nonsevere, severe, or very severe aplastic anemia (VSAA) enrolled in this study were ineligible for allo-hematopoietic stem cell transplantation (HSCT) due to a lack of a human leukocyte antigen (HLA)–matched sibling donor, being above 40 years of age, or for financial reasons. Therefore, they received p-ATG with CsA as first-line IST. AA was diagnosed based on the International Agranulocytosis and Aplastic Anemia Study Group criteria,[21] and AA severity was determined using the modified criteria described by Camitta and Bacigalupo.[22,23] Patients with congenital bone marrow failure were excluded from the study. Patients diagnosed with aplastic anemia with del 20/20q, +8, and −Y were included in this study.

Treatments

Antihuman T lymphocyte immunoglobulin administration. Patients received p-ATG for five consecutive days (day 1–5), at a dose of 20 mg/kg/day, as an intravenous infusion lasting 12–18 h. To prevent p-ATG-related serum sickness, corticosteroids were administered at a dose of 1 mg/kg/day for at least 7 days, tapered, and stopped within 2–3 weeks post-p-ATG treatment. For obese patients, p-ATG was dose adjusted according to the average between 1.3 × the ideal body weight and their actual weight.

CsA dosing and adjustments

CsA was administered orally from day 1 of p-ATG treatment at two separate doses starting from 3 to 5 mg/kg/day and adjusted to maintain the trough concentration at 150–250 ng/ml and the peak concentration at 800–1000 ng/ml. CsA was administered at the optimal dose until patients achieved the best HR for at least 3 months, and was then slowly tapered at a dose of 25–50 mg every 3 months.

Supportive care

Granulocyte colony-stimulating factor (G-CSF) was administered at a dose of 5–10 μg/kg when the absolute neutrophil count (ANC) was $<0.5\times10^9$/l and discontinued when ANC was $\geqslant0.5\times10^9$/l. Red blood cell transfusion was administered to maintain hemoglobin levels ⩾70 g/l in elderly patients. Prophylactic platelet transfusion was administered to patients with stable AA with a platelet count of $<10\times10^9$/l. Platelet transfusion was administered to patients with fever or sepsis when the platelet count was $<20\times10^9$/l. During p-ATG treatment, the platelet count was maintained at 20×10^9/l. All patients

were not given any antibacterial, antiviral, or antifungal prophylaxis after ATG administration.

Hematologic response evaluation

Complete response (CR) was defined as the fulfillment of all the following criteria without clonal evolution: hemoglobin >100 g/L, absolute neutrophils $>1.0 \times 10^9$/l, and platelets $>100 \times 10^9$/l. Partial response (PR) was defined as all the following: hemoglobin >70 g/l, absolute neutrophils $>0.5 \times 10^9$/l, platelets $>20 \times 10^9$/l, and transfusion independence. Good partial response (GPR) was defined as all the following: hemoglobin >80 g/l, absolute neutrophils $>1.0 \times 10^9$/l, platelets $>50 \times 10^9$/l, and transfusion independence. No response (NR) was defined as not meeting any of the response criteria defined above. The HRs were classified as NR if the patients died or were lost to follow-up within 6 months. Four and 14 patients were lost to follow-up within 3 and 6 months, respectively.

Follow-up and clonal evolution

The final follow-up in this study was in April 2022. Early death, delayed HR, overall survival (OS), event-free survival (EFS), clinical relapse, clonal evolution, and hemolytic paroxysmal nocturnal were evaluated during follow-up. Paroxysmal nocturnal hemoglobinuria (PNH) clones were screened every 3 months during the first year after IST and then every 6 months. Chromosomal karyotyping was performed by bone marrow aspiration at 3, 6, and 12 months during the first year after IST and then semiannually or annually to screen for clonal evolution. Definitions of these events are provided in the Supplementary Information. To assess relapse rate, patients were included regardless of the response time. Clinical relapse was defined as a decrease in blood counts to values requiring red blood cell and platelet transfusions or reinstitution of IST or HSCT. Thrombopoietin receptor agonist (TPO-RA), such as eltrombopag, was unavailable in China before 2019; thus, all nonresponse patients without rescue treatment, such as HSCT or HD-CTX, did not receive TPO-RA treatment.

Statistical analysis

All the included patients had a telephonic follow-up. The final follow-up date was 1 April 2022. SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA) was used to generate the figures. The chi-square test was used for categorical variables, and the Mann–Whitney *U* test was used for continuous variables. OS and EFS were estimated using the Kaplan–Meier method. The log-rank test was used to compare the OS and EFS values in the subgroups. The cumulative incidences of relapse and clone evolution events were estimated using a competing risk model. Statistical significance was set at $p < 0.05$.

Results

Patients

One thousand twenty-three aplastic anemia were included in this study, with a median age of 24 (4–75) years, with 550 males and 473 females. The median follow-up was 57.2 months (3 days–137.5 months). The clinical features were also noted (Table 1).

Early death

Twenty-nine patients died within 3 months after IST, with an early death rate of 2.8% (29/1023); the median death time was 0.9 (0.1–2.9) months. There were 24 patients with VSAA and 5 with SAA, with infection and hemorrhage being the main causes.

Adverse events associated with p-ATG

The common adverse events were infusion-related reactions and serum sickness. The incidence of infusion-related reactions was 3.3% during the course of p-ATG infusion, including 12 cases of fever and 21 cases of mild rashes. The incidence of serum sickness was 55.4%, including 25.7% with fever, 16.1% with rashes, and 41.0% with muscle and joint pain. The incidence of other abnormal conditions was 7.9%, including hepatotoxicity (3.0%), renal insufficiency (1.4%), arrhythmia (0.4%), hoarseness (0.9%), and hemolytic anemia (2.2%). These events were controlled by a sufficient dose of glucocorticoid treatment.

Hematologic response

The overall hematologic response rate (ORR) was 40.7% (416/1023) at 3 months, including 5.9% (60/1023) patients with CR and 12.6%

Table 1. Characteristics of the patients at baseline.

Characteristics	All patients (*N* = 1023)
Follow-up, months, median (range)	57.2 (0.1–137.5)
Age, years, median (range)	27 (4–75)
Age category, *n* (%)	
<10 years	46 (4.5%)
⩾10 to <18 years	224 (21.9%)
⩾18 to <40 years	455 (44.5%)
⩾40 to <65 years	279 (27.3%)
⩾65 years	19 (1.9%)
Sex, male/female	550/473
Severity of aplastic anemia, *n* (%)	
Very severe	379 (37%)
Severe	483 (47.2%)
Transfusion-dependent nonsevere	161 (15.7%)
Complete blood count	
Hemoglobin, g/l, median (range)	60 (35–139)
Reticulocyte count, $\times 10^9$/l, median (range)	10.4 (0.2–98.3)
Neutrophil count, $\times 10^9$/l, median (range)	0.3 (0–3.24)
Platelet count, $\times 10^9$/l, median (range)	8 (0–35)
Cytogenetic abnormalities, *n* (%)	
Normal	932 (91.1%)
Abnormal karyotype[a]	30 (2.9%)
Karyotypic analysis failed	61 (6.0%)
PNH clone positive, *n* (%)	189 (18.5%)

PNH, paroxysmal nocturnal hemoglobinuria.
[a]The category of abnormal karyotypes included 7 patients with deletion Y, 8 patients with trisomy 8, 3 patients with deletion 20 or 20q, 1 patient with deletion Y and trisomy 8, and 11 patients with other abnormal karyotypes (Supplementary Table S1).

(129/1023) with GPR. Of the 608 patients who did not respond at 3 months, 129 had an HR at 6 months. The ORR was 53.2% (544/1023) at 6 months, including 13.5% with CR and 18.9% with GPR (Figure 1).

The overall response rate at 3 months was lower in patients with VSAA (24%, 91/379) than in those with SAA (46.6%, 225/483) and tansfusion-dependent nonsevere aplastic anemia (TD-NSAA) (61.5%, 99/161) (both $p < 0.000$). The percentage of patients with CR at 3 months was 3.7% for VSAA, 6.2% for SAA, and 9.9% for TD-NSAA ($p = 0.096$, 0.000; Figure 2(a)). At 6 months, the ORRs were 38.8%, 59.4%, and 68.3% for VSAA, SAA, and TD-NSAA, respectively. The ORR was significantly lower in the VSAA group than in the other severity groups ($p < 0.000$, 0.000). The CR rate at 6 months was 9.8% for VSAA, 15.1% for SAA, and 17.4% for TD-NSAA (Figure 2(b)).

The ORR of very young (<10 years old) patients at 3 months was 60.9%, which was significantly higher than that of the other four age groups (Table 2, $p < 0.05$). The CR rate at 3 months was 13.0% in the younger group and 7.1%, 4.6%, and 6.1% in the other three age groups (<65 years). No patient achieved a CR in the oldest age group (⩾65 years) at 3 months. At 6 months, the ORR was 71.7% in the younger group, which was higher than that in the other age groups (50.9%, 57.8%, 46.2%, and 26.3%). The CR rate at 6 months was not different among age groups, except for the oldest age group (⩾65 years) (Table 2).

The ORR of patients with PNH clones at 3 months was 42.3%, with CR + GPR rate of 21.2%, which were not different from those of patients without PNH clones (ORR of 40.3% and CR + GPR rate of 18.0% at 3 months). At 6 months, the ORR and CR + GPR were 54.5% and 39.7%, respectively, for patients with PNH clones, which were similar to those of patients without PNH clones (ORR of 52.9% and CR + GPR of 30.7%). According to the year of IST, the ORR and CR rates were comparable between 2010–2014 and 2015–2019 at both 3 and 6 months (Supplementary Table S2).

Delayed HR

Of the 395 patients without early relapse and clonal evolution and who did not respond at 6 months, 26.1% (103/395) had an overall response at 12 months without any rescue treatment (10 had a CR, 30 had a GPR, and 63 had a PR). Moreover, 61.5% (243/395) of the patients had persistent NR, without any rescue treatment or clonal evolution. The other 49 patients underwent HSCT, died, or had clonal evolution. Patients with a delayed HR at 12 months had more VSAA

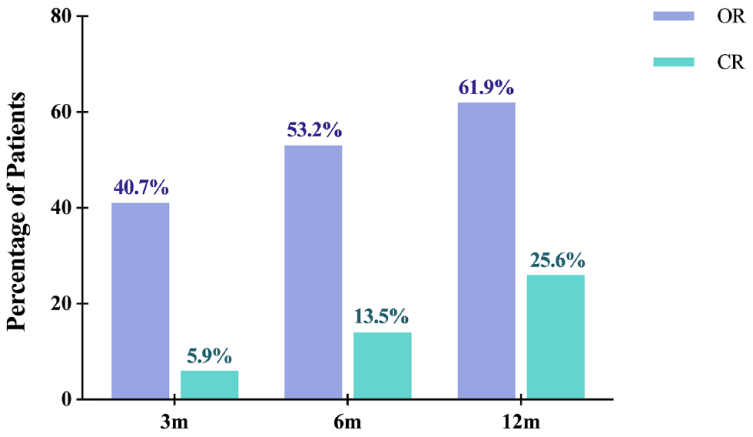

Figure 1. Hematologic response. The bar graphs showed the overall and complete responses at 3, 6, and 12 months, respectively.
OR, overall response; CR, complete response.

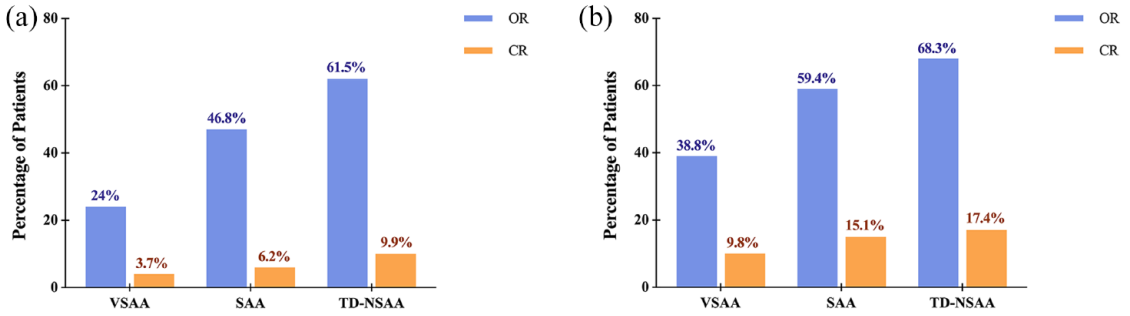

Figure 2. Hematologic response rate at 3 and 6 months (a and b) in different severity of aplastic anemia group. The bar graphs showed OR and CR at 3 and 6 months for 1023 patients in three severity groups (379 VSAA, 483 SAA, and 161 TD-NSAA), respectively.

and lower baseline median ANC, hemoglobin (HGB) levels, platelet (PLT) counts, and absolute reticulocyte count (ARC) compared with patients who had an HR within 6 months (Supplementary Table S3).

Of the 243 patients without a response at 12 months, 37.0% (90/243) had an overall response at the final follow-up, including 29 with CR, 30 with GPR, and 31 with PR. Only 48 patients were alive, with NR or any other event. Therefore, the overall delayed response rate without rescue treatment was 48.9% (193/395) at the final follow-up.

Predicting factors for HR

Details of the univariate analysis are provided in Supplementary Table S4. The multivariable analysis showed that disease history, disease severity, and age were the only three factors associated with an HR at 6 months (listed in Supplementary Table S5). Patients with older age (⩾40 years), VSAA, and longer disease history had a lower overall response at 6 months.

Rescue treatment and relapse

At the final follow-up, 60 (12.5%, 60/479) patients with NR underwent allo-HSCT as rescue treatment (48 with CR, 2 with GPR, and 10 died due to GVHD or severe infections during the HSCT), including three patients within 6 months and 20 patients within 12 months (one patient died due to graft-versus-host disease (GVHD) and 19 patients had a CR after allo-HSCT). The mortality rate associated with HSCT was 16.7% (10/60). The other

Table 2. Hematologic response, according to age.

Response and cohort	Response at 3 months	*p* value	Response at 6 months	*p* value	Response at 12 months	*p* value
Overall response, *n* (%)						
<10 years	28 (60.9)		33 (71.7)		33 (71.7)	
⩾10 to <18 years	85 (37.9)	0.004	114 (50.9)	0.010	137 (61.2)	0.177
⩾18 to <40 years	196 (43.1)	0.021	263 (57.8)	0.067	305 (67.0)	0.517
⩾40 to <65 years	103 (36.9)	0.002	129 (46.2)	0.001	150 (53.8)	0.023
⩾65 years	3 (15.8)[a]	0.001	5 (26.3)[a]	0.001	8 (42.1)[a]	0.025
Complete response, *n* (%)						
<10 years	6 (13.0)		7 (15.2)		10 (21.7)	
⩾10 to <18 years	16 (7.1)	0.184	32 (14.3)	0.870	59 (26.3)	0.937
⩾18 to <40 years	21 (4.6)	0.016	67 (14.7)	0.929	131 (28.8)	0.311
⩾40 to <65 years	17 (6.1)	0.089	32 (11.5)	0.469	57 (20.4)	0.839
⩾65 years	0	–	0	–	5 (26.3)	0.693

p value shows the difference of the overall response and complete response rate between very younger (<10 years) patients and each other four age group (compared with each two groups).
[a]The OR in very older (⩾65 years) patients was significantly lower at 3, 6, 12 months than each other four age group.

nonresponse patients at 6 months were not administered HSCT as rescue treatment due to unavailability of suitable donors or failure to afford the high cost of HSCT.

Six hundred and ninety-seven patients had an HR during the treatment period. The median follow-up time was 64.0 (10.6–137.5) months. By the follow-up time point, CsA had been discontinued in 45.4% of those who had a response. The median duration of CsA use was 39.3 (18.6–120.8) months. Moreover, 9.2% (64/697) of the patients had experienced a relapse. The percentage of patients with relapse who had a response again after reuse or adjustment of the dose of the immunosuppressive agent was 48.4% (31/64), including six with CR after HSCT, two with CR after the second course of IST, and three with CR after moderate dose cyclophosphamide. Thirty-three patients were in a constant state of NR, even with the use of CsA, and 12 (36.4%, 12/33) died due to hemorrhage and infection. The 5-year cumulative incidence of relapse was 9.0% [95% confidence interval (CI) = 4.2–16.0%] (Figure 3(a)).

Karyotypic abnormalities, myeloid cancers

The clinical and chromosomal data were available for 959 patients at the final follow-up. Therefore, these patients were enrolled for clonal evolution. The median follow-up time was 57.3 (6.2–137.5) months, the clonal evolution rate was 4.5% (43/959), and the median evolution time was 19.6 (2.9–78.8) months. Of the 43 patients, 20 had confirmed karyotypic abnormalities that met the definition of karyotypic evolution without myeloid cancers, 19 progressed to myelodysplastic syndrome (MDS) (17 cases had karyotypic abnormalities), and 4 had acute myeloid leukemia (AML). The 5-year cumulative incidence of clonal evolution was 4.5% (95% CI = 1.4–10.6%) (Figure 3(b)).

Hemolytic paroxysmal nocturnal hemoglobinuria

The incidence of hemolytic paroxysmal nocturnal hemoglobinuria (hPNH) was 3.5% (34/959), and 82.4% of cases occurred in PNH clone (+) patients prior to the IST. The 5-year cumulative incidence of hPNH was 4.7% (95%

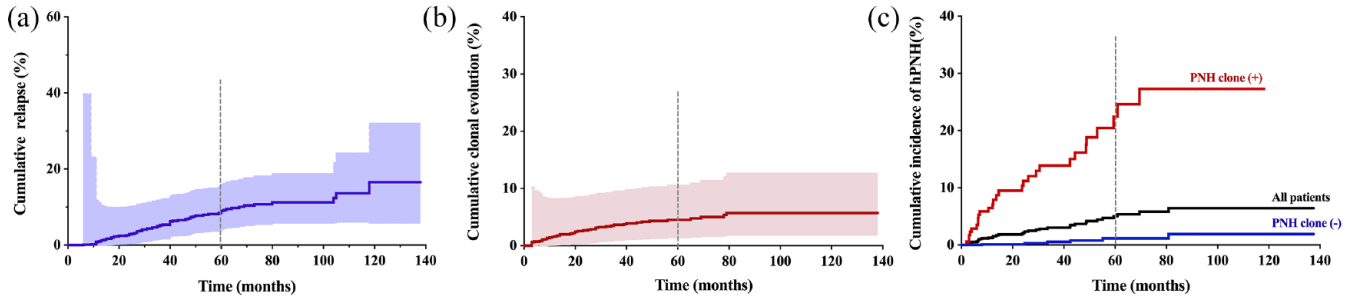

Figure 3. Cumulative incidence of relapse, clonal evolution, and hPNH. The shaded areas indicate 95% confidence intervals. Graph (a) showed that the 5-year cumulative incidence of relapse was 12.2% (95% CI = 7.1–18.9%). Graph (b) showed that the 5-year cumulative incidence of clonal evolution was 4.5% (95% CI = 1.4–10.6%). Graph (c) showed that the 5-year cumulative incidence of hPNH in all patients was 4.7% (95% CI = 1.2–12.4%). The red line indicates the cumulative incidence of hPNH in patients with PNH clones at diagnosis. The 5-year cumulative incidence of hPNH in PNH clone-positive patients at diagnosis was 22.5% (95% CI = 10.1–37.8%).

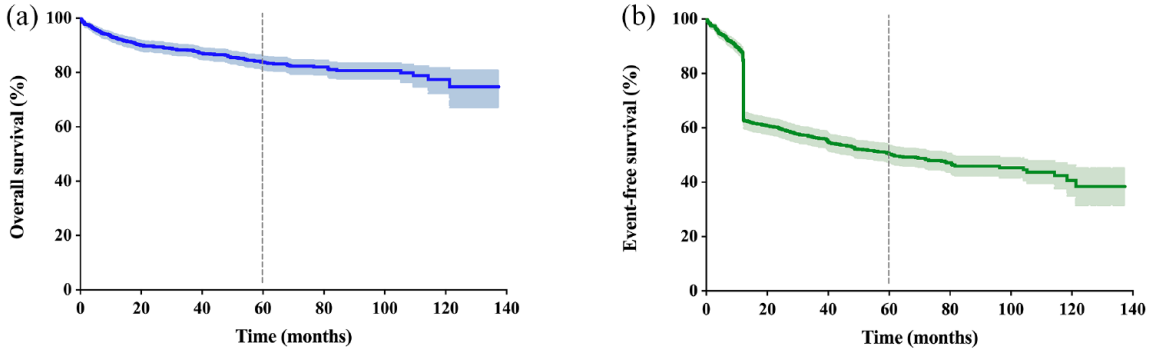

Figure 4. The 5-year overall survival and event-free survival. Graph (a) showed that the 5-year OS was 83.7% (95% CI = 81.1–86.0%). Graph (b) showed that the 5-year EFS was 50.4% (95% CI = 47.1–53.5%).

CI = 1.2–12.4%). In patients with PNH clones at diagnosis, the 5-year cumulative incidence of hPNH was 22.5% (95% CI = 10.1–37.8%) (Figure 3(c)).

Long-term outcomes

The 5-year OS rate was 83.7% (95% CI = 81.1–86.0%) (Figure 4(a)), while the 5-year EFS rate was 50.4% (95% CI = 47.1–53.5%) (Figure 4(b)).

Compared with the OS according to AA severity, the 5-year OS was similar regardless of AA severity [90.8% (95% CI = 84.6–94.6%) in TD-NSAA, 86.9% (95% CI = 83.3–89.9%) in SAA, and 77.0% (95% CI = 72.2–81.0%) in VSAA, Figure 5(a)]. In the ⩾65-year-old group, the 5-year OS of 68.4% (95% CI = 42.8–84.4%, $p < 0.001$, Supplementary Figure S1) was significantly lower than that of the other age groups [86.2% (95% CI = 71.7–93.6%) in those aged <10 years, 89.8% (95% CI = 84.8–93.3%) in those aged 10–18 years, 84.1% (95% CI = 80.1–87.4%) in those aged 18–40 years, and 78.5% (95% CI = 72.5–83.3%) in the 40–65 years group].

According to the HR at 6 months, the 5-year OS was 95.9% (95% CI = 93.5–97.4%) and 77.3% (95% CI = 72.4–81.5%) in the HR and NR groups, respectively ($p < 0.001$, Figure 5(b)). The OS was comparable between the 2010–2014 and 2015–2019 groups [84% (95% CI = 79.5–87.6%) and 83.4% (95% CI = 80–86.3%, $p = 0.685$), respectively] (Supplementary Figure S2).

Discussion

Over the past five decades, from the introduction of ATG/ALG in the 1970s to the addition of CsA to ATG in the 1980s, a standard intensive

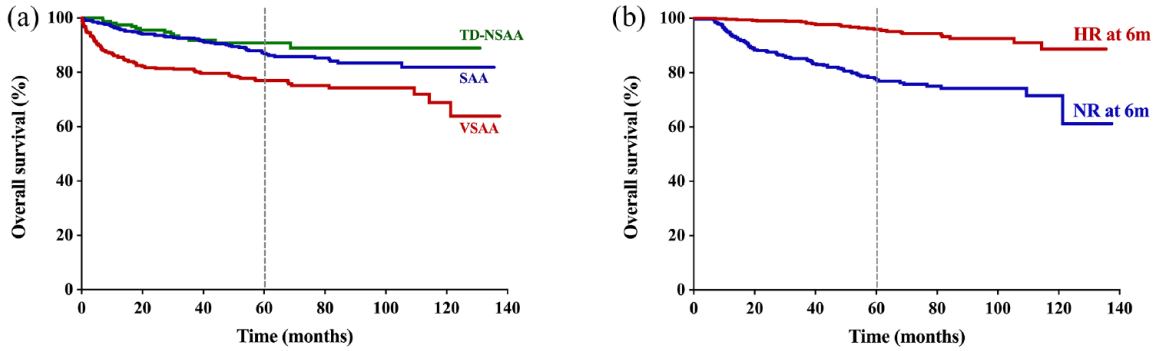

Figure 5. Graph (a) showed that the 5-year overall survivals were similar in different severity of AA groups ($p > 0.05$); 90.8% (95% CI = 84.6–94.6%) in TD-NSAA, 86.9% (95% CI = 83.3–89.9%) in SAA, and 77.0% (95% CI = 72.2–81.0%) in VSAA. Graph (b) showed that the 5-year OS was significantly lower in no response at 6 months group [77.3% (95% CI = 72.4–81.5%)] than that in hematologic response group [95.9% (95% CI = 93.5–97.4%), $p < 0.001$].

immunosuppressive regimen of ATG combined with CsA was established. There are different series of ATG preparations available, with h-ATG recommended as the first-line preparation for treating AA.[24] Unfortunately, h-ATG is unavailable in China, and some previous studies have suggested that rabbit ATG is inferior to h-ATG.[11–15] For Chinese patients with SAA, p-ATG has been the most widely used ATG preparation for the IST regimen in this decade, mainly owing to its recent efficacy, safety, and relatively low financial burden. Our previous study[25] on the pharmacokinetic characteristics of p-ATG showed that the blood concentration of p-ATG increased rapidly after administration, with a mean $t_{1/2}$ of (10.479 ± 8.242) days. During the administration of p-ATG, the absolute lymphocyte count (ALC) decreased rapidly, with a median ALC of 0.31×10^9/l at the time of peak concentration of p-ATG and the lowest value being only 16.8% of the baseline value of ALC. Besides, the T_{max} of p-ATG was similar to that of h-ATG reported in China and abroad, with $T_{1/2}\beta$ of 12.353 days, which is shorter than that of r-ATG (21.56–29.67 days). The residual drug of p-ATG was detected *in vivo* at 60 days after administration, which was shorter than that of r-ATG (90 days). The patients' lymphocytes increased quickly with the clearance of p-ATG and returned to baseline level with the stabilized concentration of p-ATG, which differs from reports that r-ATG has a longer period of lymphocyte inhibition. The immunosuppression characteristics of p-ATG were similar to those of h-ATG.[25] This study aimed to evaluate the efficacy of the IST regimen based on p-ATG for patients with aplastic anemia, and the results showed that p-ATG had not only good short-term efficacy but also favorable long-term outcomes in patients with AA.

Our results showed that the overall response rate was 53.2% at 6 months, with an early death rate of 2.8%. A prospective randomized trial from the National Institutes of Health (NIH) showed that the overall response rate was 68% at 6 months and indicated that h-ATG was superior to r-ATG in AA.[13] Results from other studies, such as that of the European Bone Marrow Transplant Society (EBMT),[14] were in accordance with those of the NIH. Recently, EBMT findings indicated that the overall response rate of h-ATG (ATGAM, Pfizer, New York, NY, USA) plus CsA was 41% and 66% by the EBMT and the NIH criteria for HR, respectively.[26] Some studies, however, have suggested that it is comparable between r-ATG (approximately 65% of OR at 6 months) and h-ATG. Our previous results also showed an overall response rate of 60.3% at 6 months.[27–30] The results of the study from Peking Union Medical College Hospital showed that the overall response rate could be as high as 83% after p-ATG combined with CsA treatment, which may be related to bias associated with the small sample size of 48 patients.[16] In addition, our and other research centers have demonstrated that the efficacy of p-ATG is comparable or superior to that of r-ATG in adults and children.[17–19,31] The comparison of the outcomes of different preparations of ATG is listed in Table 3.[11–18,29,30,32–38] Hence, although h-ATG is not available in China, the early efficacy of p-ATG is comparable to that of r-ATG or h-ATG, and the early death rate is low.

Table 3. Comparison of the overall response of different preparation of ATG.

References	Year	Study Design	ATG (N)	ORR (6 m)	Relapse rate	Clonal evolution	OS
Zheng *et al.*[11]	2006	Prospective, randomized	h-ATG (47)	78.7%	–	–	91%
			r-ATG (32)	53.2%			78%
Atta *et al.*[15]	2010	Retrospective	h-ATG (42)	59.5%	36%	–	2-year, 78.4%
			r-ATG (29)	34.5%	30%		2-year, 55.4%
Chang *et al.*[32]	2010	Retrospective	h-ATG (29)	52%	–	–	4-year, 75%
			r-ATG (33)	48%			
Afable *et al.*[12]	2011	Retrospective	h-ATG (67)	58%	16%	17%	5-year, 64%
			r-ATG (20)	45%	5%		
Scheinberg *et al.*[13]	2011	Prospective, randomized	h-ATG (60)	68%	3-year, 28%	3-year, 21%	3-year, 96%
			r-ATG (60)	37%	3-year, 11%	3-year, 14%	3-year, 76%
Marsh *et al.*[14]	2012	Prospective	h-ATG (105)	67%	–	–	2-year, 86%
			r-ATG (35)	40%			2-year, 68%
Shin *et al.*[29]	2013	Retrospective	h-ATG (46)	39.1%	3-year, 22.7%	3.03%	5-year, 83.5%
			r-ATG (53)	45.3%	3-year, 27.3%		5-year, 82.7%
Yoshimi *et al.*[33]	2013	Retrospective	h-ATG (96)	65%	–	–	3-year, 92%
			r-ATG (32)	34%			3-year, 92%
Jeong *et al.*[34]	2014	Retrospective	h-ATG (297)	60%	–	4.4%	2-year, 96%
			r-ATG (158)	55%		0.6%	2-year, 87%
Vallejo *et al.*[27]	2015	Retrospective	h-ATG (62)	75.4%	–	–	1-year, 88.1%
			r-ATG (169)	73.2%			
Zhang *et al.*[30]	2015	Retrospective	r-ATG (292)	60.3%	4.4%		5-year, 83.2%
Wei *et al.*[17]	2015	Retrospective	p-ATG (69)	73.9%	–	1.4%	2-year, 88.4%
Liu *et al.*[18]	2015	Retrospective	p-ATG (43)	66.7%	4.65%	4.65%	2-year, 87.4%
			r-ATG (32)	61.3%	6.25%	3.13%	2-year, 83.2%
Suzuki *et al.*[28]	2016	Retrospective	h-ATG (25)	56.0%	12.5%	–	2-year, 70.1%
			r-ATG (22)	64.6%	26.7%		2-year, 68.9%
Chen *et al.*[35]	2016	Retrospective	p-ATG (102)	79.4%	–	9.9%	5-year, 81.8%

(Continued)

Table 3. (Continued)

References	Year	Study Design	ATG (N)	ORR (6 m)	Relapse rate	Clonal evolution	OS
Chen *et al.*[36]	2018	Retrospective	p-ATG (114)	74.6%	–	–	5-year, 80.5%
			r-ATG (34)	64.7%			5-year, 74.1%
Cui *et al.*[37]	2018	Retrospective	p-ATG (50)	64%	10-year, 10%	6%	10-year, 80%
Hu *et al.*[38]	2022	Retrospective	r-ATG (542)	62.4%	10-year, 10.6%	10-year, 7.5%	10-year, 80.1%

ATG, antihuman thymocyte globulin; h-ATG, horse antihuman thymocyte globulin; ORR, overall hematologic response rate; OS, overall survival; p-ATG, antihuman T lymphocyte porcine immunoglobulin; r-ATG, rabbit antihuman thymocyte globulin.

Patients with SAA are in a life-threatening state; therefore, any treatment is based on keeping the patient alive. With the development of bone marrow transplantation or IST, the survival rate of patients with SAA has improved from 20–25% to 60–80%. In our large cohort with long-term follow-up, the 5-year OS was 83.7%, which was a favorable result and comparable to that of a recent EBMT study.[39] The 5-year OS was higher in pediatric patients with AA, which was in accordance with the results of Nishikawa *et al.*[40] and those of our pediatric therapy center.[41] Although the 5-year EFS was only 50.4% compared with the OS, this result indicated that more patients with NR could be alive, which is attributed to the partial improvement in blood counts after IST and intensive supportive care (including transfusion and broad-spectrum antibiotics).

Surprisingly, about 26% of patients without an HR at 6 months had a response between 6 and 12 months without rescue therapy due to personal reasons, accounting for 10.1% of the total number and 15.9% of those with a response. Whether patients with aplastic anemia acquire an HR after IST depends on the presence of immune pathogenesis, the intensity of the immunosuppressive regimen, and the number of residual hematopoietic cells. Our results showed that there was more VSAA, indicating that the number of residual hematopoietic cells was less in patients with a delayed HR. Thus, these patients may need more time to recover hematopoiesis after IST. To now, however, we do not recommend this therapeutic strategy. TPO-RAs, such as eltrombopag and hetrombopag, which can stimulate the proliferation and differentiation of hematopoietic stem/progenitor cells, have been approved for refractory SAA and have shown good efficacy;[42,43] meanwhile, these drugs can be obtained in China. Hence, applying TPO-RAs could be a rescue therapy regimen for patients with refractory aplastic anemia for IST, who were unsuitable for HSCT.

Late disorders are of special concern in IST, including relapse and clonal hematopoietic disorders such as PNH, MDS, and AML. This is an inevitable problem that needs to be addressed. It, however, does not reduce the value of IST in patients with SAA who are ineligible for HSCT. With our long-term follow-up, the 5-year cumulative incidence of relapse was only 9.0%, which was lower than that reported in most Western studies. We believe that the slow tapering of CsA contributed to the low relapse rate. For the other concerning issue, our results showed that the 5-year cumulative incidence of clonal evolution to MDS/AML was 4.5%, which was lower than the previously reported rate of 10–15%[44] but comparable to the transformation rate of our previous r-ATG study. From this point of view, the late clonal evolution of AA after IST may not be related to ATG preparations and is still caused by its own hematopoietic pressure. Therefore, regular monitoring of chromosome karyotypes remains crucial for patients with AA who are treated with IST. The main limitation of this study is being a single-center retrospective study. In future, prospective, randomized controlled clinical trials on h-ATG will be needed in China.

Conclusion

The p-ATG combined with CsA for the treatment of AA was effective and safe, and p-ATG

could be used as an alternative ATG preparation for the standard IST regimen in areas in which h-ATG is not available.

Declarations

Ethics approval and consent to participate

The studies involving patients were reviewed and approved by the Ethics Committee of the Institute of Hematology and Blood Diseases Hospital (IIT2021008-EC-1), Chinese Academy of Medical Sciences and Peking Union Medical College. Written informed consent to participate in this study was provided by patients or their guardians.

Consent for publication

Not applicable.

Author contributions

Wenrui Yang: Data curation; Formal analysis; Resources; Software; Writing – original draft.

Xu Liu: Data curation.

Xin Zhao: Data curation; Funding acquisition.

Li Zhang: Data curation.

Guangxin Peng: Data curation; Funding acquisition.

Lei Ye: Data curation.

Kang Zhou: Data curation.

Yuan Li: Data curation.

Jianping Li: Data curation.

Huihui Fan: Data curation.

Yang Yang: Data curation.

Youzhen Xiong: Data curation.

Liping Jing: Formal analysis; Writing – review & editing.

Fengkui Zhang: Writing – review & editing.

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Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The data sets used and/or analyzed during this study are available from the corresponding author on reasonable request.

ORCID iD

Wenrui Yang https://orcid.org/0000-0001-9650-3937

Supplemental material

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Yang *et al.*
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Hetrombopag plus porcine ATG and cyclosporine for the treatment of aplastic anaemia: early outcomes of a prospective pilot study

Wenrui Yang†, Xin Zhao†, Xu Liu, Youzhen Xiong, Huihui Fan, Li Zhang, Jianping Li, Lei Ye, Kang Zhou, Yuan Li, Yang Yang, Guangxin Peng, Liping Jing* and Fengkui Zhang

Abstract

Hetrombopag, a small molecular thrombopoietin-receptor agonist, has shown encouraging efficiency in immunosuppressive therapy refractory or relapsed severe aplastic anaemia. To investigate the response rate of hetrombopag combined with IST as first-line treatment, we designed a prospective pilot study including 32 patients with SAA treated with anti-human T lymphocyte porcine immunoglobulin (p-ATG), cyclosporine, and hetrombopag. In addition, 96 patients with SAA treated with p-ATG and cyclosporine alone were matched as controls. In total, 21.9% of patients treated with hetrombopag achieved complete response (CR) at 3 months, while 5.2% of patients achieved CR in the control group ($P=0.005$). At 6 months, the CR rates were 34.4% in the hetrombopag group and 14.6% in the control group ($P=0.015$). The overall response rates at 6 months were 68.7% and 50.0% in the hetrombopag and control groups, respectively. The median time to haematologic response was 56 days and 77 days, and to CR was 96 days and 214 days in the hetrombopag and control groups, respectively. In conclusion, adding hetrombopag to IST as first-line treatment resulted in faster and better haematologic response in SAA.

Keywords Hetrombopag, Aplastic anaemia, Immunosuppressive therapy

To the editor,

Aplastic anaemia (AA), a bone marrow failure disease, develops from T cell-mediated haematopoietic stem cell destruction [1]. Its response rate to standard immunosuppressive therapy (IST) is 60–70%, and the long-term overall survival is approximately 80% [1, 2]. Previously, improving the efficacy of IST was a challenge until its co-administration with eltrombopag (EPAG) was investigated. EPAG, an oral non-peptide thrombopoietin-receptor agonist (TPO-RA), was initially used in refractory severe AA (SAA) and showed an unexpected response rate of 40–50% [3, 4]. Consequently, two prospective studies showed earlier complete response (CR) and higher CR rates when EPAG plus standard IST was used in treatment-naïve SAAs [5, 6]. Currently, standard IST plus EPAG is the first-line choice for patients with SAA who are ineligible for haematopoietic stem cell transplantation (HSCT) [7].

Hetrombopag (HPAG), another oral non-peptide TPO-RA, has superior efficacy to EPAG [8]. In an open-label,

†Wenrui Yang and Xin Zhao contributed equally to this work

*Correspondence:
Liping Jing
jingliping@ihcams.ac.cn
State Key Laboratory of Experimental Hematology, Haihe Laboratory of Cell Ecosystem, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 288 Nanjing Road, Heping District, Tianjin 300020, China

non-randomized, prospective study, HPAG showed an overall response of 40% in IST-refractory SAAs [9]. It was approved for IST-refractory SAA by the China Food and Drug Administration in 2021 [10]. However, the efficacy of HPAG plus IST as first-line treatment for SAA is currently unclear; therefore, herein, we investigated this regimen at the Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences. The methods of this study were described in Additional file 1.

Patient characteristics

The median age in the HPAG group was 44 (13–69) years, with 17 males and 15 females. The median follow-up time was 366 (295–449) days in the HPAG group. The time of the last follow-up was >12 months in the control group. Patient clinical characteristics are listed in Table 1.

Haematologic response

At 3 months, the OR rates were 46.9% and 37.5% in the HPAG and control groups, respectively ($P=0.350$). The CR rate was 21.9% in the HPAG group and 5.2% in the control group ($P=0.005$) (Fig. 1A). At 6 months, the OR rates were 68.7% and 50.0% in the HPAG and control groups, respectively ($P=0.066$). The CR rate was 34.4% in the HPAG group and 14.6% in the control group ($P=0.015$) (Fig. 1B). For disease severity, a higher CR rate was observed at 3 months (23.8% vs. 6.7%) and 6 months (42.9% vs. 16.7%) in patients with SAA who received HPAG. However, the OR rate was similar between both groups. HPAG addition did not affect the OR and CR rates in patients with VSAA (Additional file 1: Table S1).

Time to response

The median time to first response was 56 days in the HPAG group and 77 days in the control group ($P=0.000$) (Additional file 1: Fig. S1A). The median time to CR was 96 days in the HPAG group and 214 days in the control group ($P=0.019$) (Additional file 1: Fig. S1B). The median time to achieving platelet counts of 100×10^9/L was 88 days in the HPAG group and 207 days in the control group ($P=0.030$).

Safety and follow-up

HPAG was well tolerated, and no patient discontinued therapy because of side effects. Seven patients discontinued HPAG owing to no response at 6 months, and eight stopped owing to platelet counts. One patient had a relapse of AA after discontinuing HPAG. One patient had disease relapse during therapy and HPAG was stopped. HPAG was tapered in five patients and maintained at a dose of 15 mg in eight patients. Platelet counts decreased to $<100\times10^9$/L in two patients with CR who tapered HPAG to discontinuation. Nonetheless, they re-achieved CR after re-initiating treatment at a dose of 7.5 mg (Fig. 1C).

By the last follow-up, the OR and CR rates were 65% and 43.8%, respectively. Two patients had a relapse of AA, and no patient died within 6 months after IST in the HPAG group. However, one patient died during the HSCT course (Fig. 1C); there were no complications, including haemolytic paroxysmal nocturnal haemoglobinuria and myelodysplastic syndromes/acute myeloid leukemia.

Discussion

EPAG plus standard IST has been shown to improve response rate and quality in SAA [5, 6]. HPAG has comparable efficacy as EPAG in IST-refractory SAA [9]. Therefore, we conducted this study to determine its use as a first-line treatment. HPAG plus IST significantly increased the CR rate. The HR rate increased by approximately 10% and 20% in the HPAG group

Table 1 Clinical characteristics at baseline

	Hetrombopag group (n = 32)	Control group (n = 96)	*P* value
Age (years, median, range)	44 (13–69)	45 (7–70)	0.928
Gender (male/female)	17/15	52/44	0.919
Severity of aplastic anaemia, n (%)			
Severe	21 (66%)	60 (63%)	0.752
Very severe	11 (34%)	36 (37%)	
Complete blood count (median, range)			
Reticulocyte count ($\times10^9$/L)	15.2 (0.2–68.4)	12.9 (0–63.7)	0.821
Neutrophil count ($\times10^9$/L)	0.33 (0–1.04)	0.33 (0–1.12)	0.739
Platelet count ($\times10^9$/L)	7 (1–21)	8 (0–31)	0.361
Haemoglobin level (g/L)	55 (34–99)	61 (33–96)	0.046
PNH clones (+), n (%)	11 (34%)	26 (27%)	0.433

Hetrombopag group: IST plus hetrombopag; control group: IST alone

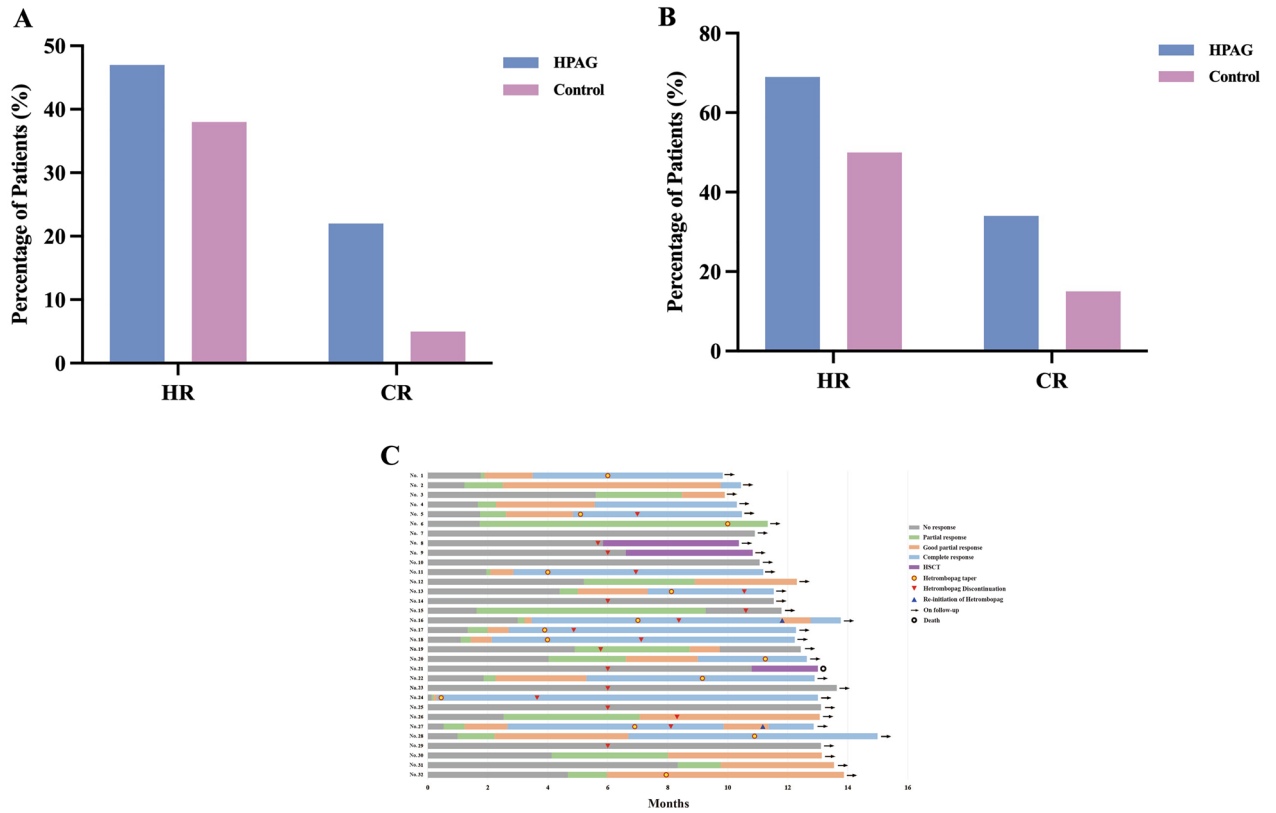

Fig. 1 Graph A shows that the HR and CR rates at 3 months were 46.9% vs. 37.5% and 21.9% vs. 5.2%, respectively, in the two groups. Graph B shows that the HR and CR rates at 6 months were 68.7% vs. 50.0% and 34.4% vs. 14.6%, respectively, in the two groups. Graph C shows the haematologic responses over time in each patient. In total, 23 patients had a response after hetrombopag with IST treatment. However, patients No. 15 and No. 19 had disease relapse, one owing to discontinued hetrombopag, and the reason was unknown for the other. Patients No. 16 and No. 27 had fluctuating platelet counts of $< 100 \times 10^9$/L after discontinuing hetrombopag; however, their platelet counts returned to normal after re-initiation of hetrombopag at a dose of 7.5 mg. Only one patient died during HSCT at 393 days after IST. *HR* haematologic response, *CR* complete response

compared to the control group at 3 and 6 months, respectively. Nevertheless, the difference was not significant owing to the small sample size. HPAG plus IST improved haematologic and CR rates in patients with SAA or VSAA, especially the CR rate in patients with SAA, which is consistent with RACE study results.

The National Institutes of Health study showed a cumulative relapse rate of 39% in patients with SAA treated with IST plus EPAG, compared to IST alone [11]. Herein, two patients experienced relapse which may be associated with dose tapering of cyclosporine and HPAG. The median follow-up time of this study was relatively short; thus, we could only determine the early outcomes. Further follow-up is needed to analyse long-term outcomes. This is just a pilot study, with a prospective, randomized, double-blind, placebo-control clinical trial (NCT04961710) ongoing in China to confirm these results.

HPAG plus IST, compared with IST alone, was beneficial in patients with SAA and induced a higher quality and faster haematologic response without increasing adverse events.

Abbreviations

AA	Aplastic anaemia
IST	Immunosuppressive therapy
EPAG	Eltrombopag
HPAG	Hetrombopag
SAA	Severe aplastic anaemia
TPO-RA	Thrombopoietin-receptor agonist
CR	Complete response
HSCT	Haematopoietic stem cell transplantation
VSAA	Very severe aplastic anaemia
PR	Partial response
OR	Overall response

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40164-023-00377-3.

Additional file 1. The details of the methods, including study design, treatment protocol, haematologic response criteria, and statistical analysis. The predicting factors for haematologic response for IST plus hetrombopag. Table S1 showed haematologic response according to severity of aplastic anemia.

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Author contributions
WY and XZ collected and analysed the data, performed statistical review and literature review, and wrote and revised the manuscript; XL collected and analysed the data; YX, HF, JL, LZ, LY, KZ, YL, YY, and GP enrolled patients and oversaw the human subject aspects of the research; LJ and FZ conceived and oversaw the study; and all authors edited the final version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The ethics committee of the Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences, approved this study. All patients or their guardians provided written informed consent in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors have no competing interests.

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· 论著——临床研究 ·

抗人 T 细胞猪免疫球蛋白联合艾曲泊帕治疗初发重型再生障碍性贫血疗效分析*

宫跃敏[1] 马永超[2] 陈小玉[1] 李瑞鑫[1] 李悦[1] 张雅文[1] 师锦宁[2] 龙启强[3]
杨岩[4] 贾晋松[5] 林圣云[6] 何广胜[1] 李建勇[1]

［摘要］ 目的：研究抗人 T 细胞猪免疫球蛋白(anti-human T lymphocyte porcine immunoglobulin，p-ATG)，环孢素 A(Cyclosporin A，CsA)组成的强化免疫抑制治疗(intensive immunosuppressive therapy，IST)联合艾曲泊帕在重型再生障碍性贫血(severe aplastic anemia，SAA)患者的疗效及影响因素。方法：前瞻性登记纳入 2020 年 4 月—2022 年 8 月 25 例初发 SAA 患者，接受 p-ATG 为基础的 IST 联合艾曲泊帕治疗。主要终点是 6 个月完全反应(complete response，CR)率和总体反应率(overall response rate，ORR)。次要终点是生存率、复发率及克隆演变风险。结果：治疗后 6 个月 CR 率和 ORR 分别为 19.05%、71.43%。中位获得反应时间为 3.00(0.10～11.80)个月，中位获得 CR 时间为 14.40(0.90～20.00)个月，多因素分析显示疾病严重程度对获得反应时间有显著影响。中位随访时间 11.30(0.70～26.40)个月，总生存率 96.00%，中位无事件生存期为 12.70(1.40～18.90)个月。1 例于治疗后 10 个月出现克隆性造血。≥3 级不良反应：感染 8 例，1 例死亡；肝肾功能损害 1 例和牙周病 2 例。结论：p-ATG 为基础的 IST 联合艾曲泊帕治疗 SAA 可获得较为满意的血液学反应率和反应速度，耐受性和安全性良好。

［关键词］ 重型再生障碍性贫血；抗人 T 细胞免疫球蛋白；艾曲泊帕
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Anti-human T-cell porcine immunoglobin combined with eltrombopag in patients with severe aplastic anemia

GONG Yuemin[1] *MA Yongchao*[2] *CHEN Xiaoyu*[1] *LI Ruixin*[1] *LI Yue*[1]
ZHANG Yawen[1] *SHI Jinning*[2] *LONG Qiqiang*[3] *YANG Yan*[4]
JIA Jinsong[5] *LIN Shengyun*[6] *HE Guangsheng*[1] *LI Jianyong*[1]

([1]Department of Hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing, 210029, China; [2]Department of Hematology, the Affiliated Jiangning Hospital of Nanjing Medical University; [3]Department of Hematology, Second Hospital of Nanjing, the Affiliated Nanjing Hospital of Nanjing Traditional Chinese Medical University; [4]The Hematology & Tumor Center of the First Hospital of Jilin University; [5]Department of Hematology, People's Hospital of Peking University; [6]Department of Hematology, Zhejiang Traditional Chinese Medicine Hospital)

Corresponding author: HE Guangsheng, E-mail: heguangsheng1972@sina.com

Abstract **Objective**: To evaluate the efficacy and safety of anti-human T-cell porcine immunoglobin(p-ATG) based intensive immunosuppressive therapy(IST) combined with eltrombopag in patients with severe aplastic anemia(SAA). **Methods**: From April 2020 to August 2022, 25 newly-diagnosed SAA patients enrolled in a prospec-

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[1]南京医科大学第一附属医院 江苏省人民医院血液科(南京，210029)
[2]南京医科大学附属江宁医院血液内科
[3]南京市第二医院 南京中医药大学附属南京医院血液科
[4]吉林大学第一医院血液肿瘤中心
[5]北京大学人民医院血液科
[6]浙江省中医院血液科
通信作者：何广胜，E-mail：heguangsheng1972@sina.com

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tive cohort registry study were treated by p-ATG plus eltrombopag. The primary outcome was complete response (CR) and overall response rate(ORR) at 6 months. Secondary end points included survival, relapse, and clonal evolution. **Results**: CR and ORR at 6 months were 19.05% and 71.43%, respectively. The median time to first response was 3.00(0.10-11.80) months. The median time to achieve complete response was 14.40(0.90-20.00) months. Severity (vSAA vs SAA) was associated with the time to get first response. The median follow-up was 11.30(0.70-26.40) months, the over survival rate was 96.00%. The median event-free survival time was 12.70 (1.40-18.90) months. Clonal hematopoiesis was found in one patient 10 months after initiation of treatment. Adverse events above grade 3 occurred in 8 cases of infection, including one death; 1 case of abnormal liver and renal functions, and 2 cases of parodontopathy, which were relieved by adjusting concentration of CsA. **Conclusion**: The addition of EPAG to p-ATG based IST was well tolerant and associated with high rates of hematologic response among previously untreated patients with SAA.

Key words　severe aplastic anemia; antithymocyte globulin; eltrombopag

再生障碍性贫血是自身反应性 T 细胞破坏造血干/祖细胞导致的以血细胞减少和骨髓衰竭为特征的疾病[1-2]。抗人胸腺细胞免疫球蛋白(antithymocyte globulin，ATG)联合环孢素(cyclosporin A，CsA)组成的强化免疫抑制治疗(intensive suppressive therapy，IST)是不适合接受异基因造血干细胞移植(hematopoietic stem cell transplantation，HSCT)治疗的重型再生障碍性贫血(severe aplastic anemia，SAA)患者的一线选择，有效率达 50%～70%[3-4]。已有文献报道，兔抗人胸腺细胞免疫球蛋白(rabbit antithymocyte globulin，r-ATG)因免疫抑制强，导致感染发生率高，临床疗效差于马抗人胸腺细胞免疫球蛋白(horse antithymocyte globulin，h-ATG)联合 CsA 治疗 SAA 的效果[5-6]。猪和马相较于兔，均为更接近人的大型动物，抗人 T 细胞猪免疫球蛋白(anti-human T lymphocyte porcine immunoglobulin，p-ATG)是我国自主研发的 ATG，可能对 SAA 也有较佳的效果。

促血小板生成素(thrombopoietin，TPO)受体小分子激动剂——艾曲泊帕结合于 TPO 受体跨膜区，刺激体内残存造血干/祖细胞的扩增[7]。近年来研究表明，h-ATG 或 r-ATG 为基础的 IST 加用艾曲泊帕可显著提高 SAA 患者的反应率、反应速度和质量[8-11]。p-ATG 联合艾曲泊帕疗效如何目前尚无报道，因此在中国东部贫血协作组(Chinese Eastern Collaboration Group of Anemia，CECGA)前瞻性登记研究了接受 p-ATG 联合艾曲泊帕治疗的初发 SAA 患者疗效、安全性和相关因素，初步报告如下。

1　资料与方法

1.1　资料

2020 年 4 月—2022 年 8 月在 CECGA[包括：南京医科大学第一附属医院(江苏省人民医院)、南京医科大学附属江宁医院、南京市第二医院(南京中医药大学附属南京医院)、吉林大学第一医院、北京大学人民医院、浙江省中医院血液科]登记住院，依据英国血液学指南[12]确诊为获得性 SAA 的患者 25 例，无法接受 HSCT，选择接受 IST。入组标准：①骨髓细胞增生程度＜正常的 25%；如≥正常的 25%但＜50%，则残存的造血细胞应＜30%；②血常规需具备下列 3 项中的 2 项：中性粒细胞计数(ANC)＜0.5×10⁹/L；网织红细胞绝对值(ARC)＜20×10⁹/L；血小板计数(PLT)＜20×10⁹/L；③既往未使用过 ATG 治疗。排除标准：①先天性骨髓衰竭症，包括：范可尼贫血、先天性角化不良症、先天性无巨核细胞性血小板减少症、先天性纯红细胞再生障碍性贫血；②临床典型的阵发性睡眠性血红蛋白尿；③细胞遗传学提示克隆性髓系疾病，包括骨髓增生异常综合征(MDS)。根据赫尔辛基宣言及药品临床试验管理规范(GCP)，经伦理委员会批准开展了前瞻性登记研究(ChiCTR2100045895)，患者本人或其监护人均已签署知情同意书。

1.2　IST 方案

p-ATG 30 mg/(kg·d)静脉输注，维持 12～18 h，连用 5 d；同时给予泼尼松 1 mg/(kg·d)预防过敏反应和血清病，2 周后泼尼松开始减量，4 周停药。患者疾病诊断明确后即开始 CsA 口服，以 3 mg/(kg·d)分 2 次口服起始，1 周后检测血药浓度，调整 CsA 剂量维持全血谷浓度 200～300 ng/mL。口服 CsA 至少 12 个月，后根据血液学参数在 6～12 个月内缓慢减停，总体服药时间 2～3 年。

1.3　艾曲泊帕

艾曲泊帕以 25 mg/d 开始口服，每 3 d 增量 25 mg，1 周内增至 75 mg/d 并维持至少 6 个月。剂量调整方案：PLT＞400×10⁹/L，停药 1 周，待 PLT 恢复至＜100×10⁹/L 在原剂量基础上减量 25 mg/d 服药；PLT≥100×10⁹/L 但≤400×10⁹/L，每 2 周减量 25 mg 维持 PLT≥50×10⁹/L；PLT≥50×10⁹/L、脱离输血超过 8 周，减量 50%；减量后 PLT≥50×10⁹/L、脱离输血超过 8 周，停药；减量后 PLT＜50×10⁹/L、血红蛋白浓度(HGB)＜90 g/L 或 ANC＜0.5×10⁹/L，重新服用原有效剂量。

1.4 观察终点

主要终点：治疗 6 个月内不良反应，参照常见不良事件评价标准（CTCAE）5.0 版。治疗 6 个月完全反应（complete response，CR）率和总体反应率（overall response rate，ORR）。

次要终点：治疗 3、6、12 个月部分反应（partial response，PR）率；生存率；复发率；阵发性睡眠性血红蛋白尿（PNH）克隆；克隆演变，定义为新出现细胞遗传学异常克隆，或骨髓象转化为 MDS 或急性髓系白血病（AML）。

1.5 随访和疗效评估

随访截止日期为 2022 年 9 月 30 日。患者在获得治疗反应前每周至少 2 次血常规检测。所有患者均于 IST 后 3、6、12 个月进行血液学随访评估治疗反应，此后每年随访 1 次，观察包括外周血和骨髓细胞形态学及组织活检、细胞遗传学、髓系肿瘤基因突变、PNH 克隆、脏器功能、免疫指标等以评估血液学反应、不良反应和克隆演变。疗效评价参考 RACE 试验[9] 标准：①HGB≥100 g/L、ANC≥1.0×10^9/L 且 PLT≥100×10^9/L 判定为 CR；②脱离红细胞和血小板输注，血液学参数不再符合 SAA 标准而未达 CR 标准者判定为 PR；③未达 PR 标准者为无治疗反应（NR）。治疗失败定义为 IST 后 6 个月仍未获得治疗反应；复发定义为血液学指标下降至需要输血、需要再次 IST 或 HSCT 治疗。无事件生存期（EFS）定义为开始 p-ATG 至治疗失败、复发、死亡、转行 HSCT、失访或随访截止。

1.6 统计学处理

使用 SPSS 23.0 软件进行统计学分析，正态分布计量资料以 $\overline{X}\pm S$ 表示，采用独立 t 检验；非正态分布计量资料以 $M(P_{25},P_{75})$ 或中位数（范围）表示，使用 Mann-Whitney U 检验。计数资料以例（%）表示，采用 χ^2 检验。使用 R 4.2.1 软件绘制累积反应率和生存分析曲线。单因素分析采用非参数检验或 χ^2 检验，多因素分析采用广义线性模型或 Cox 回归分析。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 基线临床特征

25 例患者基线临床资料见表 1。21 例患者治疗前获得分子遗传学资料，其中存在髓系肿瘤基因突变者 4 例，突变基因包括 *DNMT3A*、*RUNX1*、*ARID1A*、*U2AF1*。

2.2 疗效分析

治疗后不同时间点疗效见表 2。截至末次随访，ORR 72.00%（18/25），CR 率 32.00%（8/25）。

3 个月和 6 个月累积反应率分别为（51.70±10.40）%和（85.50±7.70）%，中位获得反应时间为 3.00（95%*CI* 2.82～3.18）个月（图 1a）。3、6、12 个月累积完全反应率分别为（12.70±7.70）%、（17.80±8.20）%和（32.80±11.70）%，中位获得 CR 时间为 14.40（95%*CI* 11.09～17.71）个月（图 1b）。中位随访 11.30（0.70～26.40）个月，6 个月和 12 个月 EFS 率分别为（73.50±9.30）%、（56.50±11.20）%（图 1c），OS 率为（96.00±3.90）%（图 1d），仅 1 例 77 岁 SAA 患者在治疗后 1 个月内死于重症感染。2 例于治疗后 6 个月复发，其中 1 例为感染诱发，另 1 例因肾功能损害导致环孢素减量诱发，这 2 例截至随访终点仍为 NR。

表 1 25 例 SAA 患者基线临床资料

基线临床特征	数值
年龄/岁	24（3～77）
性别/例	
女	11
男	14
严重程度/例	
SAA	16
vSAA	9
诊断至 ATG 治疗的时间/d	27.00（3.00～639.00）
治疗前感染/例	13
PNH 克隆/例	2
铁蛋白/（ng/mL）	885.70（523.82～1 139.59）
HGB/（g/L）	68.00（59.00～72.00）
ANC/（×10^9/L）	0.30（0.09～0.45）
淋巴细胞计数/（×10^9/L）	1.31（0.43～1.85）
PLT/（×10^9/L）	12.00（5.50～15.50）
ARC（×10^9/L）	10.20（5.55～32.35）
红细胞分布宽度变异系数（RDW-CV）/%	14.80（12.10～16.60）
$CD3^+$ T 细胞比例/%	72.03（81.46～84.40）
$CD4^+$ T 细胞/%	47.30（31.40～57.75）
$CD8^+$ T 细胞/%	24.00（18.55～35.50）
调节性 T 细胞/%	5.30（4.58～7.06）
髓系肿瘤基因突变/例	4

2.3 影响疗效的因素

结合文献报道的 SAA 接受 IST 治疗反应预测因素[13-17]，对基线临床参数对重要疗效指标的影响进行了单因素分析，结果显示，影响 6 个月 ORR 的因素有：年龄（$P=0.039$）、疾病严重程度（$P=0.047$）、淋巴细胞计数（$P=0.032$）和 CD4/CD8 T 细胞的比值（$P=0.009$）。

影响获得反应时间的因素有：疾病严重程度（$P=0.001$）、ANC（$P=0.006$）、ARC（$P<0.001$）、RDW（$P=0.002$）、淋巴细胞计数（$P=0.009$）和 HGB（$P=0.021$）。其中，vSAA 患者获

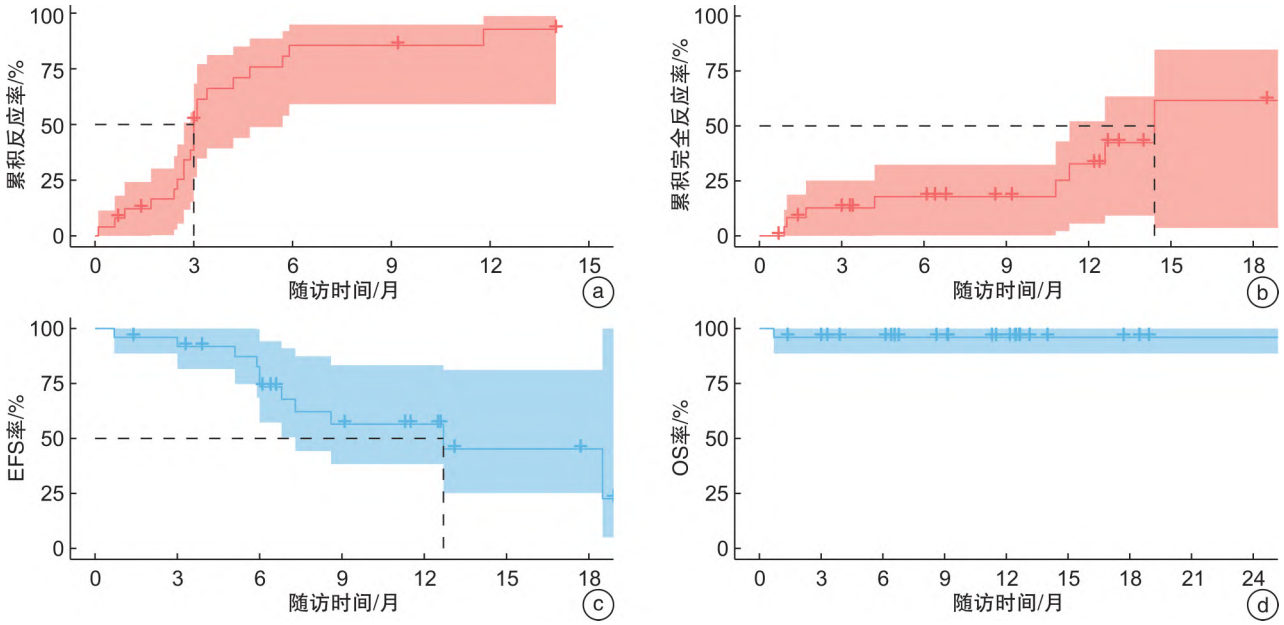
得反应的中位时间为 5.9(95%*CI* 5.4～6.4)个月，SAA 患者中位反应时间仅 2.9(95%*CI* 2.6～3.2)个月。

影响 EFS 的因素有：$CD3^+$ T 细胞比例(P=0.017)和 CD4/CD8 T 细胞比值(P=0.006)。

表 2　p-ATG 联合艾曲泊帕治疗 SAA 不同时间点疗效

治疗时间	1 个月	3 个月	6 个月	12 个月
CR/例	1	3	4	5
PR/例	2	12	11	4
NR/例	22	9	6	4
死亡/例	0	1	1	1
不可评估*/例	0	1	4	12
ORR/%	12.00(3/25)	62.50(15/24)	71.43(15/21)	69.23(9/13)
CR 率/%	4.00(1/25)	12.50(3/24)	19.05(4/21)	38.46(5/13)

*失访或未达评估时间。



a：累积反应率；b：累积完全反应率；c：EFS 率；d：OS 率。

图 1　p-ATG 为基础的 IST 联合艾曲泊帕治疗 SAA 累积疗效曲线和生存曲线

2.4　不良反应

≥3 级不良反应包括：新发感染或原有感染加重 8 例，除 1 例进展为脓毒症死亡外，其余均通过积极抗感染和支持治疗后控制；肝、肾功能损害 1 例，牙周病 2 例，考虑与 CsA 有关，调整 CsA 剂量后好转。25 例患者中 3 例出现发热、皮疹、心悸、水肿等 1～2 级输注反应，3 例发生 2 级血清病，经对症处理后好转。

截至末次随访尚未发现向 MDS、AML 转化的病例；1 例于治疗后 10 个月出现 *BCOR p.K1098** 和 *IDH1 p.R132H* 突变，变异等位基因频率(variant allele fraction，VAF)分别为 30.15%、1.03%，提示克隆性造血。

3　讨论

ATG 联合 CsA 组成的 IST 是无同胞全相合供者或年龄＞40 岁的 SAA 患者一线治疗方案[3,18-19]。目前临床上应用的 ATG 种属来源有兔(r-ATG)、猪(p-ATG)和马免疫球蛋白(h-ATG)。前瞻性随机对照试验表明，h-ATG 的疗效优于 r-ATG，显著提高了患者的 6 个月 ORR(68.00% vs 37.00%)和 3 年生存率(96.00% vs 76.00%)[5]。回顾性研究显示，h-ATG 相比 r-ATG 提高了患者的长期生存率[6]。究其原因可能与 r-ATG 免疫抑制效果更强，导致患者早期更易发生感染有关。猪和马相较于兔，均为更接近人的大型动物，药代动力学研究显示 p-ATG 血药浓度达峰时间与 h-ATG相似，消除相半衰期和体内残留时间明显短于 r-ATG，患者淋巴细胞恢复也较快，不同于 r-ATG较长时间的免疫抑制，而与 h-ATG 免疫抑制特征相似[20]。h-ATG国内目前尚无，而 p-ATG

作为我国自主研发和广泛应用的同类产品，治疗SAA可能也有较好的效果[21]。

由于不同人种药代动力学差异，艾曲泊帕在亚洲人群的推荐剂量为 75 mg/d，仅为西方 SAA 患者治疗剂量(150～300 mg/d)的一半[8]。美国国立卫生院(NIH)的研究提示艾曲泊帕与 IST 同时开始疗效优于 IST 14 d 后加用艾曲泊帕，且艾曲泊帕延长使用(从 3 个月延长至 6 个月)有助于提高 CR 率[8,22]。因此，我们在 p-ATG 同时合用艾曲泊帕，持续至少 6 个月，治疗剂量用 75 mg/d。相比基于 h-ATG的 RACE 研究，患者经 p-ATG 联合艾曲泊帕治疗后 3 个月和 6 个月 ORR 与 RACE 试验 ORR(59.00%和 68.00%)相仿[9]；3 个月和 6 个月 CR 率低于 RACE 试验的 22.00% 和 32.00%，但末次随访时 CR 率达到了 32.00%。相比我们基于 r-ATG 的真实世界研究数据[10]，p-ATG联合艾曲泊帕治疗 6 个月累积反应率与 r-ATG(85.00%)相当，累积完全反应率低于 r-ATG(27.00%)，但末次随访时 CR 率与之相仿。

这组患者的中位获得 CR 时间为 14.40 个月，明显长于 RACE 试验(约 9 个月)，提示这组患者可达到相当的 CR 率，但获得 CR 时间较迟。62.50%～71.43%的患者在治疗后 3～6 个月获得反应，此后 ORR 增长不明显；而 CR 率可持续增长至治疗后 12 个月。CR 延迟获得的趋势在 RACE 试验中有所提示[9]。在 r-ATG 为基础的 IST 联合艾曲泊帕治疗的临床观察中也发现了这种情况[10]，是不是对于亚洲人群艾曲泊帕 75 mg/d 的剂量偏小或者延长艾曲泊帕使用时间以提高 CR 率？这 2 点尚需前瞻性临床试验验证。

淋巴细胞计数是对 p-ATG 联合艾曲泊帕 6 个月治疗反应有利的因素，与 h-ATG[16]和 r-ATG[15]联合艾曲泊帕的研究报道一致；本研究发现 $CD4^+$ 和 $CD8^+$ T 细胞的比例对反应率和反应速度有影响，总体来说 $CD4^+$ T 细胞比例低者治疗反应较好、反应时间较早；鉴于 SAA 患者体内常见 $CD4^+/CD8^+$ 比值倒置，这一相关性可能提示 T 细胞免疫紊乱越明显者反应越好[14,23]。有研究指出代表残存造血功能的 ARC 和 ANC 亦与疗效相关[9-10,24]，在本研究中则主要影响早期(1～3 个月)治疗反应。

3 级以上感染在这组患者中发生率 32.00%(8/25)，与 NIH 报道的 h-ATG 联合艾曲泊帕的临床试验相仿(35.00%)[8]，高于 RACE 试验的感染率(5.20%)[9]，而死于重症感染的比例(4.00%)与 RACE 试验相仿(4.17%)。血清病发生率 12.00%(3/25)，稍高于 NIH 的报道(7.00%)，但 3 例均未超过 3 级，程度轻于 NIH 的研究(3 级以上 7.00%)[8]。肝功能损害发生率 20.00%(5/25)，3 级以上 4.00%(1/25)，低于 RACE 试验报道的 35.42%和 7.29%，与 NIH 的报道相仿(18.00%)。总体而言，p-ATG 联合艾曲泊帕方案的安全性与 h-ATG 联合艾曲泊帕大致相当。而相比 r-ATG 联合艾曲泊帕较高的输注反应和血清病发生率(84.00%和 26.00%)[10]，以及较高的早期感染风险(65.00%)[10]，p-ATG 显然作用更温和，早期不良反应更少。肝肾功能损害发生率则与 r-ATG联合艾曲泊帕(28.00%)[10]相仿。

本研究中位随访 11.3 个月，OS 率 96.00%，与 NIH 的研究(97.00%)[8]、RACE 研究(90.00%)[9]以及基于 r-ATG 的真实世界研究数据(98.00%)[10]相仿；患者 6 个月和 12 个月 EFS 率亦与 RACE 试验(79.00%和 56.00%)相当[9]。治疗 6 个月后，15 例获得反应的患者中有 2 例(13.33%)复发，低于 NIH 的报道的 32.00%[8]，可能与 NIH 的队列中患者在 6 个月后停用艾曲泊帕且 CsA 开始减量[25]有关，提示延长艾曲泊帕的使用或可减少早期复发，有待进一步前瞻性研究验证。鉴于 2 个基于 h-ATG 的研究中位随访时间均达到 24 个月，本方案的长期生存和复发情况仍需继续随访观察。

本研究患者治疗前髓系肿瘤突变基因的检出率(19.05%)低于 RACE 研究(31.00%)；RACE 研究提示具有髓系突变基因克隆的患者 3 个月 CR 率和 6 个月 ORR 可能有优势[9]，这一点在我们的队列研究中差异不明显，可能与该组患者年龄较小、突变阳性病例数较少有关。RACE 试验报道患者克隆性造血在治疗后呈现频率增多趋势，提示克隆性造血和 SAA 患者治疗后血常规的恢复可能有关。本研究亦观察到 1 例无突变患者治疗后 10 个月出现 *BCOR* 基因突变克隆，*BCOR* 突变在 RACE 和 NIH 研究中均是 SAA 患者治疗前后高频出现的克隆[9,26]。克隆性造血对 SAA 患者 IST 后反应的意义尚需进一步探究。

NIH 研究(中位年龄 32 岁)在长达 4 年的随访中观察到 15.00%的患者发生细胞遗传学克隆演变，5.70%的患者出现 7 号染色体缺失、复杂核型等提示向 MDS、AML 转化的高危克隆，甚至最终确诊为 MDS、AML[13]。与单用 IST 组相比，艾曲泊帕联合治疗组克隆演变发生率差异无统计学意义，但发生演变的中位时间要明显短于单用 IST(186 d vs 777 d)[26]，最早于治疗后 3 个月就可观察到高危克隆演变[8]。本研究中截至随访终点尚未观察到转化为 AML、MDS 的病例，是否与队列年龄较小(中位年龄 24 岁)，还是艾曲泊帕剂量更低有关？尚需进一步观察研究，以确定艾曲泊帕的使用时间和剂量对于 SAA 患者髓系肿瘤转化的风险。

综上，基于 p-ATG 的 IST 联合艾曲泊帕治疗 SAA 可获得较为满意的血液学反应率和反应速度，而不良反应较低。但本研究纳入的患者尚少，随访时间也相对较短，有待于进一步扩大病例数和延长随访时间，乃至进行前瞻性对照研究，以明确该方案的临床效果，包括克隆演变的可能情况。

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·论著·

按需糖皮质激素治疗对再生障碍性贫血p-ALG治疗相关血清病反应发生及转归的影响

杨夏婉 周康 李建平 樊慧慧 杨文睿 叶蕾 李园 李洋
彭广新 杨洋 熊佑祯 赵馨 井丽萍 张莉 张凤奎
中国医学科学院血液病医院(中国医学科学院血液学研究所),实验血液学国家重点实验室,国家血液系统疾病临床医学研究中心,细胞生态海河实验室,天津 300020
通信作者:张凤奎,Email:fkzhang@ihcams.ac.cn

【摘要】 目的 探讨按需糖皮质激素治疗对再生障碍性贫血(AA)应用猪抗人淋巴细胞球蛋白(p-ALG)相关血清病反应发生及转归的影响。**方法** 收集2019年1月至2022年1月连续就诊于中国医学科学院血液病医院贫血诊疗中心接受p-ALG治疗的AA患者资料,其中按需治疗组患者根据血清病反应发生与否及严重程度应用糖皮质激素,共35例;传统防治组患者从同期接受常规糖皮质激素防治方案的群体中依据年龄、疾病诊断分型按1∶3进行倾向性评分匹配,共纳入105例。比较两组间血清病发生率、临床表现、治疗转归及糖皮质激素用量。**结果** 按需治疗组和传统防治组患者的血清病发生率分别为65.7%和54.3%,差异无统计学意义($P=0.237$)。两组患者血清病中位发病时间相同[第12(9, 13)天对第12(10, 13)天,$P=0.552$];临床症状体征相似,主要表现为关节和(或)肌肉疼痛、发热及皮疹;严重程度分级均以1~2级血清病反应为主(62.8%对51.4%),仅少数3级反应(2.9%对2.9%),无4级和5级血清病反应,不同等级血清病反应在两组间分布差异无统计学意义($P=0.530$);血清病中位持续时间相同[5(3, 7)d对5(3, 6)d,$P=0.529$],均经糖皮质激素治疗后完全缓解。在未发生血清病反应的患者中,传统防治组平均每例患者的糖皮质激素预防量为(469.48±193.57)mg(按需治疗组预防量为0)。在发生血清病反应的患者中,按需治疗组平均每例患者的糖皮质激素治疗量显著少于传统防治组[(125.91±77.70)mg对(653.90±285.56)mg,$P<0.001$]。**结论** 按需治疗策略可显著减少糖皮质激素用量,而并不增加血清病发生率,血清病持续时间和2级以上血清病反应发生率较传统防治组患者相近。

【关键词】 贫血,再生障碍性; 血清病; 糖皮质激素

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The effect of on-demand glucocorticoid strategy on the occurrence and outcome of p-ALG-associated serum sickness in aplastic anemia

Yang Xiawan, Zhou Kang, Li Jianping, Fan Huihui, Yang Wenrui, Ye Lei, Li Yuan, Li Yang, Peng Guangxin, Yang Yang, Xiong Youzhen, Zhao Xin, Jing Liping, Zhang Li, Zhang Fengkui
State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China
Corresponding author: Zhang Fengkui, Email: fkzhang@ihcams.ac.cn

【Abstract】 Objective To investigate the effect of on-demand glucocorticoid strategy on the occurrence and outcome of porcine anti-lymphocyte globulin (p-ALG)-associated serum sickness in aplastic anemia (AA). **Methods** The data of AA patients who received in the Anemia Diagnosis and Treatment Center of Haematology Hospital, CAMS & PUMC from January 2019 to January 2022 were collected. Among them, 35 patients were enrolled in the on-demand group, with the glucocorticoid strategy adjusted based on the occurrence and severity of serum sickness; 105 patients were recruited in the

usual group by matching the age and disease diagnosis according to 1∶3 ratio in patients who received a conventional glucocorticoid strategy in the same period. The incidences, clinical manifestations, treatment outcomes of serum sickness, and glucocorticoid dosage between the two groups were analyzed. **Results** The incidences of serum sickness in the on-demand group and the usual group were 65.7% and 54.3% ($P=0.237$), respectively. The median onset of serum sickness was the same [12(9, 13)d *vs* the 12(10, 13) d, $P=0.552$], and clinical symptoms and signs, primarily joint, and/or muscle pain, fever, and rash were similar. Severity grades were both dominated by Grades 1–2 (62.8% *vs* 51.4%), with only a few Grade 3 (2.9% *vs* 2.9%), and no Grades 4–5. No significant difference in the serum sickness distribution ($P=0.530$). The median duration of serum sickness was the same [5(3, 7)d *vs* 5(3, 6)d, $P=0.529$], and all patients were completely cured after glucocorticoid therapy. In patients without serum sickness, the average dosage of prophylactic glucocorticoid per patient in the usual group was (469.48±193.57) mg (0 in the on-demand group). When compared to the usual group, the average therapeutic glucocorticoid dosage per patient in the on-demand group was significantly lower [(125.91±77.70) mg *vs* (653.90±285.56) mg, $P<0.001$]. **Conclusions** In comparison to the usual glucocorticoid strategy, the on-demand treatment strategy could significantly reduce glucocorticoid dosage without increasing the incidence of serum sickness; in addition, the duration of serum sickness and the incidence of above Grade 2-serum sickness were similar.

【**Key words**】 Anemia, aplastic; Serum sickness; Glucocorticoid

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抗胸腺细胞球蛋白(ATG)和环孢素A(CsA)的联合免疫抑制治疗(IST)是不适于造血干细胞移植治疗再生障碍性贫血(AA)患者的标准一线治疗方案[1]。作为异种免疫球蛋白,传统上ATG输注期间须同步应用糖皮质激素预防即刻过敏反应,输注结束后仍需继续使用至少2~3周以预防血清病反应。在此预防基础上,一旦出现血清病,则需追加糖皮质激素用量[1]。猪抗人淋巴细胞球蛋白(p-ALG)的疗效与兔/马ATG相近[2-4],近几年在国内普遍应用,预防和治疗血清病时糖皮质激素的使用仍然沿用了和兔/马ATG一致的方案。但该方案主要基于理论和经验,并无相关前瞻性研究证据支持。长时程的糖皮质激素可能导致药物相关并发症(如糖尿病、高血压、骨病、感染等)发生率增加,同时也会进一步加重AA患者骨髓的脂肪化[5]。因此减少传统方案中糖皮质激素的用量既符合临床需求,也是个体化治疗的要求。我们采取按需治疗的方案,包括不预防性应用糖皮质激素和对发生血清病反应者根据临床表现分级调整剂量,探索这一策略对AA患者p-ALG相关血清病反应发生及转归的影响,以期了解糖皮质激素的实际预防作用并优化用药方案。

病例与方法

1. 病例选择:本研究为回顾性队列研究。收集2019年1月至2022年1月间连续就诊于我院贫血诊疗中心接受IST方案(p-ALG联合CsA)的AA患者337例,其中35例患者根据血清病反应发生与否及严重程度采取按需糖皮质激素治疗方案,其余302例患者采用传统的糖皮质激素防治方案。对按需治疗患者及传统防治患者进行倾向性评分匹配,匹配依据为患者的年龄、疾病诊断分型,设定按需治疗组∶传统防治组为1∶3、卡钳值为0.02,最终共纳入按需治疗组患者35例,传统防治组患者105例。两组患者匹配前后变量特征详见表1。

2. 血清病防治方案:两组患者接受p-ALG(武汉生物制品研究所产品)20 mg·kg^{-1}·d^{-1}静脉输注,同时给予等效泼尼松1 mg·kg^{-1}·d^{-1}剂量的糖皮质激素预防即刻过敏反应,连用5 d。CsA则以3~5 mg·kg^{-1}·d^{-1}分两次口服,维持谷浓度150~250 μg/L。传统防治组方案:p-ALG结束后继续口服等效泼尼松1 mg·kg^{-1}·d^{-1}剂量的糖皮质激素,从第15天开始减量,至第22天停药,用以预防血清病反应。当发生血清病反应时,追加等效泼尼松1 mg·kg^{-1}·d^{-1}剂量的糖皮质激素静脉滴注,症状缓解后停药。按需治疗组方案:p-ALG结束后无糖皮质激素预防用药,如发生血清病反应,则予静脉输注糖皮质激素,1~2级血清病反应患者给予泼尼松0.5 mg·kg^{-1}·d^{-1}等效剂量,3级及以上患者给予泼尼松1 mg·kg^{-1}·d^{-1}等效剂量,症状缓解后停药。

3. 血清病诊断及严重程度评估:血清病诊断和严重程度分级主要根据患者的临床表现进行

表1 按需治疗组与传统防治组再生障碍性贫血患者倾向性评分匹配前后的临床特征比较

指标	匹配前				匹配后			
	按需治疗组（35例）	传统防治组（302例）	统计量	*P*值	按需治疗组（35例）	传统防治组（105例）	统计量	*P*值
年龄[岁，*M*(*IQR*)]	43(26, 55)	34.5(24, 52)	*z*=−1.083	0.279	43(26, 55)	44(28, 56)	*z*=−0.337	0.736
疾病诊断分型[例(%)]			χ^2=6.014	0.049			χ^2=0.948	0.622
TD-NSAA	8(22.9)	64(21.2)			8(22.9)	26(24.8)		
SAA	24(68.6)	156(51.7)			24(68.6)	64(61.0)		
VSAA	3(8.5)	82(27.2)			3(8.5)	15(14.3)		

注 TD-NSAA：输血依赖非重型再生障碍性贫血；SAA：重型再生障碍性贫血；VSAA：极重型再生障碍性贫血

评估，参照文献[6-7]标准，分级如下：1级：发热（<38.5 ℃）、轻微关节或肌肉疼痛、局限性皮疹；2级：中等程度关节或肌肉疼痛，发热（≥38.5 ℃）、多部位皮疹或荨麻疹；3级：严重的多发性关节或肌肉疼痛，全身广泛性皮疹；4级：危及生命的症状；5级：死亡。

4. 相关定义：血清病发生率定义为在p-ALG治疗28 d内，发生血清病反应的患者人数占所在治疗组总人数的比例。血清病发生时间是从p-ALG起始治疗时间开始计算。血清病持续时间定义为从开始发生血清病反应至症状完全缓解的间期。糖皮质激素累积量定义为从患者完成5 d p-ALG治疗，即第6天始，至糖皮质激素结束期间的总用量（按照泼尼松剂量计，下同）的总和，其中糖皮质激素预防量定义为第6天至第22天期间用于预防血清病反应的糖皮质激素用量总和，糖皮质激素治疗量定义为从患者出现血清病反应当天始，至血清病反应完全缓解日期间全部糖皮质激素用量的总和。

5. 统计学处理：采用SPSS 22.0进行统计学分析，符合正态分布的计量资料，用均值±标准差描述，组间比较采用*t*检验，非正态分布的连续变量，用中位数（*IQR*）描述，组间比较采用Mann-Whitney *U*检验，分类变量用例数（百分比）描述，组间比较采用χ^2检验或Fisher精确概率法。*P*<0.05为差异有统计学意义。

结　果

1. 一般临床特征：共纳入按需治疗组和传统防治组140例患者，男77例，女63例，男女比为1.22∶1，中位年龄44（14～71）岁。其中，输血依赖非重型AA（TD-NSAA）34例（24.3%），重型AA（SAA）88例（62.9%），极重型AA（VSAA）18例（12.9%）；初诊治疗时伴感染者27例（19.3%），不伴感染者113例（80.7%）。两组患者性别、中性粒细胞绝对计数（ANC）、HGB、PLT、网织红细胞绝对值（ARC）、治疗时有无感染、体重的差异均无统计学意义（均*P*>0.05）（表2）。所有患者按IST方案完成5 d p-ALG输注，均无严重即刻过敏反应发生。

2. 血清病反应的发生：按需治疗组患者的血清病发生率为65.7%（23/35），中位发生时间为治疗第12（9, 13）天，其中两周内发病者22例（95.6%），超过两周发病者1例（4.4%）。传统防治组患者的血清病发生率为54.3%（57/105），中位发生时间为治疗第12（10, 13）天，其中两周内发病者51例（89.5%），超过两周发病者6例（10.5%）。比较两组患者的血清病发生率和发生时间，差异均无统计学意义（*P*值分别为0.237、0.552）。

3. 血清病表现及严重程度：按需治疗组23例发生血清病反应的患者中9例（39.1%）发热，8例（34.8%）出现皮疹，17例（73.9%）出现关节和（或）肌肉疼痛，1例（4.4%）表现为胸腔积液。传统防治组57例发生血清病反应的患者中27例（47.4%）发热，17例（29.8%）出现皮疹，43例（75.4%）出现关节和（或）肌肉疼痛，8例（14.0%）出现不同程度的腹痛、胸痛、肝肾功能异常等。两组患者出现不同临床表现的构成差异均无统计学意义（均*P*>0.05）。

按需治疗组患者中，1～2级血清病反应占62.8%（22/35），3级血清病反应占2.9%（1/35）。传统防治组中，51.4%（54/105）的患者为1～2级血清病反应，2.9%（3/105）的患者为3级血清病反应。两组均无4级和5级血清病反应。不同等级血清病反应在两组间分布差异无统计学意义（*P*=0.530）。

4. 血清病的转归：按需治疗组患者的中位血清病持续时间为5（3，7）d，其中2例（8.7%）血清病持续时间为1 d（发病当天应用糖皮质激素治疗即可获

表2　按需治疗组与倾向性评分匹配后传统防治组再生障碍性贫血患者的临床特征比较

指标	按需治疗组(35例)	传统防治组(105例)	统计量	*P*值
性别[例(%)]			χ^2=0.779	0.377
男	17(48.6)	60(57.1)		
女	18(51.4)	45(42.9)		
ANC[×10^9/L, *M*(*IQR*)]	0.51(0.40, 0.90)	0.48(0.28, 0.75)	*z*=−0.915	0.132
HGB[g/L, *M*(*IQR*)]	67(58, 81)	66(58, 76)	*z*=−0.915	0.360
PLT[×10^9/L, *M*(*IQR*)]	12(7, 21)	12(7, 17)	*z*=−0.684	0.494
ARC[×10^{12}/L, *M*(*IQR*)]	0.020(0.013, 0.044)	0.020(0.010, 0.040)	*z*=−0.664	0.506
治疗时有无感染[例(%)]			χ^2=0.015	0.902
有	7(20.0)	20(19.0)		
无	28(80.0)	85(81.0)		
体重[kg, *M*(*IQR*)]	68(59, 77)	69(56, 78)	*z*=−0.833	0.405

注　ANC:中性粒细胞绝对计数;ARC:网织红细胞绝对值

得缓解),18例(78.3%)血清病症状在1周内缓解,最长血清病持续时间为18 d。传统防治组患者的中位血清病持续时间为5(3,6)d,其中有3例(5.3%)糖皮质激素治疗当天症状缓解,47例(82.5%)于1周内症状好转,最长持续时间为14 d。两组患者的血清病持续时间差异无统计学意义(*P*=0.529)(图1)。两组患者的血清病反应症状均得到完全缓解,未观察到不可逆转损伤。

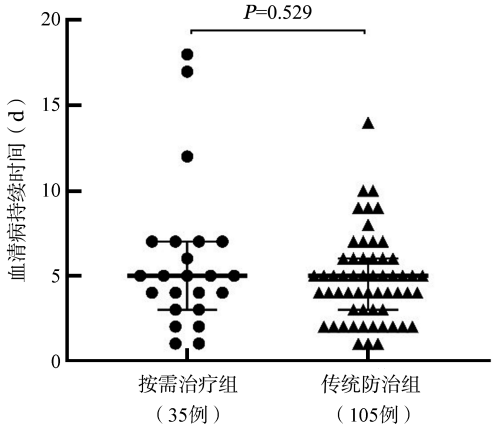

图1　按需治疗组和传统防治组再生障碍性贫血患者的血清病持续时间

5. 糖皮质激素用量:所有患者的糖皮质激素用量均按照泼尼松剂量进行计算。按需治疗组平均每例患者的糖皮质激素累积量显著少于传统防治组[(82.74±87.08)mg对(569.59±263.37)mg,*P*<0.001]。在未发生血清病反应的患者中,传统防治组平均每例患者的糖皮质激素预防量为(469.48±193.57)mg,按需治疗组预防量为0 mg。在发生血清病反应的患者中,按需治疗组平均每例患者的糖皮质激素治疗量亦显著少于传统防治组[(125.91±77.70)mg对(653.90±285.56)mg,*P*<0.001]。

讨　论

血清病的概念由von Pirquet和Schick于1905年首次提出,是一种因异种血清制品进入体内产生血循环免疫复合物而引起的Ⅲ型变态反应性疾病[8]。国内报道的ATG/ALG相关血清病发生率为52.0%~68.0%[9-12]。本研究中,按需治疗组和传统防治组患者的血清病发生率分别为65.7%和54.3%,与文献报道相近,且两组相比差异无统计学意义(*P*=0.237),提示不预防性应用糖皮质激素治疗并未增加血清病发生率。进一步分析两组中发生血清病反应患者的临床表现,血清病反应中位发生时间均为第12天,多数患者的发病时间集中在p-ALG治疗两周内(95.6%对89.5%,*P*=0.552);其症状体征相似,主要表现为关节和(或)肌肉疼痛、发热、皮疹,少见腹痛、胸痛、肝肾功能异常、体腔积液等不典型症状。以上结果显示是否给与预防性糖皮质激素不会改变血清病反应的发生时间和表现形式。两组患者的血清病反应均以1~2级的轻型血清病表现为主,仅少数3级反应(2.9%对2.9%),无4级和5级血清病反应。尽管血清病反应严重程度在两组间分布差异无统计学意义(*P*=0.530),但按需治疗组的轻症血清病反应发生率(62.8%)略高于传统防治组(51.4%),仍可能具有现实意义,我们推测预防性糖皮质激素的应用不会降低重症血清病反应的发生,可能仅是减少了轻症患者的发生。

糖皮质激素治疗血清病反应的转归包括临床表现的缓解速度和缓解质量。研究显示，按需治疗组与传统防治组发生血清病反应的患者的持续时间相近[5(3, 7)d对5(3, 6)d，$P=0.529$]，大多数患者的血清病反应都可在1周内好转(78.3%对82.5%)，表明按需给药策略不会影响血清病反应的总时程，同时我们也观察到两组患者的血清病反应症状均能得到完全缓解，无不可逆转损伤发生。

糖皮质激素是ATG/ALG治疗方案中的重要辅助性药物，但也会引起诸多不良反应发生[13]。此外对于接受IST的AA患者，由于细胞免疫受抑制，本身就存在较高的感染风险[14-15]，因此，减少IST期间不必要糖皮质激素的使用尤为重要。本研究结果显示，在未发生血清病反应的患者中，按需治疗组每例患者的平均糖皮质激素预防剂量(0 mg)较传统防治组的(469.48±193.57)mg形成鲜明对照。即使出现血清病反应，我们及时发现、及时治疗，根据不同级别的严重程度采取相应的按需糖皮质激素方案，也能显著减少糖皮质激素的治疗用量[(125.91±77.70) mg 对 (653.90±285.56) mg，$P<0.001$]。这些均表明按需治疗策略能够避免糖皮质激素的过量使用，从而减少相应并发症的出现概率。

总之，按需治疗策略可显著减少糖皮质激素用量，而并不增加血清病发生率，重型血清病发生率和血清病持续时间较传统防治组患者相比并无差别，支持p-ALG相关血清病的预防和治疗采用按需糖皮质激素方案。

本研究为回顾性观察研究，且纳入观察的部分病例随访时间较短，没有对长期糖皮质激素相关不良反应及AA疗效的影响做进一步深入研究，存在一定的局限性。此外，本研究纳入的患者均接受p-ALG治疗，研究结果是否同样适用于其他动物来源ATG制剂也需进一步探讨。

利益冲突 所有作者声明无利益冲突

作者贡献声明 杨夏婉：采集统计数据，设计方案，分析解释数据，起草文章；周康：设计方案，文章审阅；张凤奎：研究设计，对文章内容进行指导并修改审阅；其他：采集数据，审阅文章

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SYSTEMATIC REVIEW ARTICLE

Treatment of Severe Aplastic Anemia with Porcine Anti-Human Lymphocyte Globulin

Qi Lv[1], Zhang Huiqin[1], Xiao Na[1], Liu Chunyan[1], Shao Zonghong[1] and Wang Huaquan[1,*]

[1]*Department of Hematology, General Hospital, Tianjin Medical University, Tianjin 300052, China*

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Abstract: Aplastic anemia (AA) is a bone marrow failure syndrome characterized by pancytopenia. Decreased numbers of hematopoietic stem cells and impaired bone marrow microenvironment caused by abnormal immune function describe the major pathogenesis of AA. Hematopoietic stem cell transplantation and immune-suppressive therapy are the first-line treatments for AA. Porcine anti-lymphocyte globulin (p-ALG) is a new product developed in China. Several studies have shown that p-ALG exhibited good therapeutic effects in AA.

Keywords: Anemia, aplastic, porcine anti-human lymphocyte globulin, immunosuppressive, treatment, hematopoietic.

1. INTRODUCTION

Aplastic anemia (AA) is a group of diseases that are caused by chemical, physical, biological factors and unknown reasons, such as bone marrow stem cell damage and abnormal hematopoietic microenvironment, resulting in the replacement of hematopoietic bone marrow by adipose tissue and the reduction of whole blood cells. The etiology of AA has not yet been determined, but clinical experience and laboratory data showed that the most likely mechanism is cytotoxic T-lymphocyte hyperfunction and immune-mediated hematopoietic stem or progenitor cell destruction. Combined immunosuppressive therapy (IST) composed of antithymocyte globulin (ATG) and cyclosporine A (CSA) can improve the hematopoiesis of bone marrow in more than 70% AA patients, which is the most powerful indirect evidence of AA immune pathogenesis. Porcine anti-lymphocyte globulin (p-ALG) is a new product developed in China. In this paper, we reviewed the efficacy of p-ALG in AA, including response rates, survival and side effects.

2. DEFINITIONS

AA is a representative disease of bone marrow failure syndrome. The main pathogenesis of AA is the damage of hematopoietic stem cells caused by functionally and phenotypically activated cytotoxic T lymphocyte (CTL) and its secretion of cytokines as hematopoietic inhibitors. The change of the hematopoietic microenvironment caused by AA accelerates the development of the disease. The main clinical manifestations of AA are various symptoms caused by pancytopenia, such as anemia, infection and bleeding. In recent years, with the progress of IST and hematopoietic stem cell transplantation (HSCT), the cure rate and survival time of AA have been increased significantly [1-3].

3. IMMUNOPATHOGENESIS

The pathogenesis of AA includes the inherent proliferation deficiency of hematopoietic stem cells, disturbance of hematopoietic microenvironment, and disorder of immune regulation with hereditary tendency. More evidence have shown that activated CTL, increased levels of γ-interferon (IFN-γ) and tumour necrosis factor-α (TNF-α) and inadequate regulation of T cells collectively lead to the immune damage of hematopoietic stem/progenitor cells and the abnormal microenvironment of bone marrow, which recapitulates the main pathogenesis of AA [4, 5].

Numerous studies have shown that abnormal cellular immunity is the main pathogenesis of AA, which is supported by the significant efficacy of intensive immunosuppressive therapy [6-9]. Almost all severe and acute aplastic anemia seems to be immune-mediated because the blood counts respond well to immunosuppressive therapies [2, 10].

The expression of IFN-γ and TNF-α in $CD4^+$ and $CD8^+$ cells of AA patients was up-regulated compared with that of normal controls [11]. IFN-γ and TNF-α upregulate both the T cell's cellular receptors and the Fas receptor. Increased production of interleukin-2 leads to polyclonal expansion of T cells [12].

In vitro and clinical observations have identified that cytotoxic T-cell mediated attack on CD34+ multipotential hematopoietic cells as the basis for most cases of acquired aplastic anemia [12]. T cells in AA patients are in a pre-activated state and are easy to be activated by external stimuli. Activated cytotoxic T cells are skewed to produce type 1 cytokines [13, 14], which induce apoptosis through the Fas/FasL [15] pathway. In addition, regulatory T (Treg) cells are decreased in untreated AA patients and could be increased when patients acquire hematological response after treatment [16-18]. It was found that in the peripheral blood of patients with SAA, the percentage of CD4+CD25+CD127dimTregs in lymphocytes is decreased. This reflects the impaired immune tolerance in AA, which leads to T cell hyperfunction, resulting in hematopoietic failure [19].

Recent studies in bone marrow failure have shown that cells with mutations in specific genes have survival advantages [20, 21], since these cells could avoid epidemic-mediated damage (such as mutations in human leucocyte antigen (HLA) class I genes) and exhibit accelerated proliferation [18]. The study by Babushok *et al*. [20] showed that somatic mutations mainly occurred in genes regulating immunity and transcription in younger AA patients. Immune evasion is one of the main driving forces of clonal hematopoiesis.

*Address correspondence to this author at the Department of Hematology, General Hospital, Tianjin Medical University, Tianjin 300052, China;
Tel: 0086 2260817095; Fax: 0086 2260817535;
E-mail: wanghuaquan@tmu.edu.cn

Young *et al*. suggested that mutations in uniparental disomy (UPD) in 6p (6pUPD) and PIGA genes are highly specific in AA, and immune evasion may be one of the mechanisms of clonal selection [22]. 6pUPD represents the most frequent genetic damage in AA [21, 23], which is produced by the recombination of two homologous chromosomes, often involving the distal end of the short arm of chromosome 6. All 6pUPD involve HLA class I loci, which results in the uniparental expression of the retained HLA class I allele [23]. The process of unknown pathogenic antigen being presented to CTL depends on the HLA class I gene. 6pUPD-positive hematopoietic stem cells can escape CTL-mediated immune damage due to the deletion of the HLA class I gene. On the other hand, the frequent occurrence of PIGA gene mutant clones in AA patients suggests that PIGA mutant cells have a strong survival advantage [21, 24-26]. However, the potential molecular mechanism of how PIGA mutant cells escape remains unclear. At present, it is considered that the molecular biological mechanism of PNH lies in the defect of GPI-anchor protein synthesis caused by PIGA gene mutation. Some studies showed that the expansion of PNH clones *in vivo* was due to the defect of GPI anchor protein, which could avoid the immune attack of NK cells in the NGK2D pathway [27]. It is speculated that the survival advantage of PIGA mutant cells may be due to the loss of expression of GPI-anchoring protein, which is of great significance for immune recognition.

4. TREATMENTS

4.1. Hematopoietic Stem Cell Transplantation (HSCT)

Allogeneic HSCT (Allo-HSCT) is the first-line treatment for SAA patients younger than 35 years with HLA-matched family donors [28]. For AA, the long-term survival rate is more than 90% in young children [29, 30] and more than 80% in adolescents receiving allo-HSCT [31], with a low rate of short-term and long-term adverse reactions. The pathogenesis of AA is different from that of malignant hematopathy. Bone marrow stem cells are the preferred source of stem cells for HSCT because bone marrow contains not only stem cells necessary for hematopoietic recovery, but also stromal cells and other components which could assist the recovery of damaged hematopoietic microenvironment [32]. In contrast, hematopoietic stem cells from peripheral blood may lead to more Graft-*versus*-Host Diseases (GVHD) [33, 34]. As for transplantation pretreatment, anti-human thymocyte globulin (ATG), fludarabine, cyclophosphamide and other drugs with strong immunosuppressive effects are mostly used at present, with fewer adverse reactions. The combination with cyclophosphamide and ATG significantly enhances the effect of overcoming transplant rejection, and there is no risk of secondary tumors, which may occur in systemic radiotherapy [35]. Graft failure is one of the main problems of stem cell transplantation in the treatment of aplastic anemia, which has a higher incidence than that of HSCT in other hematological diseases. The main reason is that the residual immunity in patients with AA attacked and rejected the grafts, especially in the case of HLA-incompatible transplants. Other issues include the cytotoxicity of high dose cyclophosphamide pretreatment, which might be further improved in the future.

4.2. IST

HSCT from HLA-matched donors is suitable for most young patients with SAA, but less than 30% of the patients have suitable donors. Immunosuppressive therapy (IST) is highly recommended for patients older than 40, or those who do not have HLA-identical donors or not suitable for HSCT [36]. At present, the immunosuppressive agents for SAA include ATG, anti-lymphocyte globulin (ALG) and CsA. There are horse, pig and rabbit sources for ATG or ALG according to species and genus. According to the study of the National Institutes of Health (NIH) and the European Collaborative Group on Bone Marrow Transplantation (EBMT), the efficacy of horse ATG in the treatment of newly diagnosed SAA is significantly better than that of rabbit ATG. Therefore, horse ATG is the first-line treatment for SAA in the USA and Europe (but not available since 2007). Scheinberg *et al*. [18] compared the efficacy of rabbit ATG with horse ATG, which showed that the 6-month effective rate was 37% and 68%, respectively ($P < 0.001$). The 3-year overall survival rate excluding HSCT was 96% for horse ATG and 76% for rabbit ATG (P=0.004). To those including HSCT, the 3-year overall survival rate was 94% for horse ATG and 70% for rabbit ATG (P=0.008). There was no significant difference in the 3-year recurrence rate and 3-year clonal evolution rate between the two groups. The EBMT SAA Working Group conducted a multicenter, non-randomized, non-blind, prospective phase II clinical trial to evaluate the efficacy of rabbit ATG combined with CsA as a first-line treatment in 35 patients with AA. One hundred and five AA patients matched by age and severity of disease who were treated with horse ATG combined with CsA were set as a control group. None of the patients treated with rabbit ATG achieved complete remission (CR), and 11 patients (34%) achieved partial remission (PR) in 3 months. The 6-month CR rate and PR rate were 3% and 37%, respectively. Ten patients died in the rabbit ATG group, among which one died after HSCT and nine died of infection. The best effective rate in the rabbit ATG group and horse ATG group was 60% and 67%, respectively. The 2-year overall survival rate in the rabbit ATG group and horse ATG group was 68% and 86%, respectively (P=0.009). The non-transplantation survival rate in the rabbit ATG group and horse ATG group was 52% and 76%, respectively (P=0.002). Multivariate analysis showed that rabbit ATG was an independent risk factor for survival (HR = 3.9, P = 0.003) [37]. In a retrospective study of 71 patients with AA, the 6-month effective rate of patients receiving horse ATG (60%) was higher than that of patients receiving rabbit ATG (36%), and the former had a higher survival rate. In multivariate analysis, rabbit ATG was an independent predictor of death [38]. In addition, most studies in Europe and the United States have shown that the efficacy and survival rate of AA treated with rabbit ATG are low [3].

However, some clinical trials showed that the therapeutic effect of rabbit ALG was not inferior to that of horse ATG. Afable *et al*. compared 20 SAA patients treated with rabbit ATG with 67 SAA patients treated with horse ATG. The results showed that the effective rate of the rabbit ATG group and horse ATG group in 3, 6 and 12 months was similar (40% *vs*. 55%, P=0.43; 45% *vs* 58%, P=0.44; 50% *vs*. 58%, P=0.61, respectively). There was no significant difference in the early mortality rate and the total survival rate [39]. A European study also showed that there was no significant difference between the total effective rate of horse ATG (66% and 76% at 3 and 12 month) and rabbit ATG (63% and 84% at 3 and 12month) as a first-line treatment [40]. Two retrospective studies in Asia showed that rabbit ATG had similar efficacy as horse ATG [41, 42]. A total of 124 children with AA were treated with rabbit ATG combined with CsA. The overall response rate was 55.6%. The median response time was 6 months. The median follow-up period was 29 months. The recurrence rate and clonal evolution rate were 3.2% and 0.8%, respectively [3]. The 5-year overall survival rate was 74.7%.

Porcine anti-lymphocyte globulin (p-ALG) is a product developed in China. Several clinical trials in China have confirmed that its therapeutic effect in SAA is at least not inferior to rabbit ALG [43].

5. P-ALG

5.1. Mechanism

At present, mechanisms of horse-derived and rabbit-derived ATG/ALG have been studied and reported, however, the mechanism of p-ALG has not yet been clarified.

ATG is a polyclonal immunoglobulin (IgG) which contains antibodies to T cells, B cells, monocytes, natural killer cells, adhe-

sion molecules and transduction molecules [44-46]. In the presence of complement, ATGs act against an abnormal population of circulating T-suppressor cells demonstrated in some patients with AA [47]. ATG has been shown to induce apoptosis of normal peripheral blood lymphocytes, which release IFN-γ and TNF-α *via* the Fas antigen (Fas-Ag)/Fas ligand (Fas-L) interaction [48]. ATG could also induce the release of hematopoietic growth factors, resulting in *in vitro* colony stimulation [49, 50], and shows an effect on the proliferation and differentiation of normal hematopoietic progenitor cells [51, 52]. Killick *et al*. [53] reported that ATG led to an increase in bone marrow colony growth when incubated with CD34+ cells from the bone marrow of AA patients, which may represent one possible mechanism of hematopoietic recovery caused by ATG in AA.

5.2. Effect

The overall response rate of p-ALG treatment is 65%~80% [54-70] (Table **1**). Han Bing *et al*. first reported the efficacy of p-ALG (20~30mg/kg/d, d1~5) combined with CsA (3~5mg/kg/d) treatment in 48 patients with SAA from 1999 to 2014. The overall response rate (ORR) was 83.3%, complete remission (CR) rate was 54.2%, partial remission (CR) rate was 29.2%, and non-remission (NR) rate was 6.3%. The median duration from treatment to response was 90 (23~380) days. The 1.5-year survival was 87.5% [54, 55]. Mild toxicities were observed in these cases. In a later study, 102 SAA patients were enrolled to verify the long-term efficacy of p-ALG combined with CsA in the treatment of SAA. The median follow-up time was 59.6 (0.2~176.8) months. The ORR was 71.6%, 79.4% and 81.8% at 3 months, 6 months and 5 years, respectively, after IST [56]. In an extensive study, they studied naïve SAA patients treated with ATG and CsA, among which there were 114 patients in the pALG group and 34 patients in the rATG group. The overall responses (OR) were 65.8% and 44.1%, respectively (P = 0.023) in pALG and rATG groups at three months. There was a lower incidence of MDS/AML transformation in the pALG group (2.6% *vs*. 11.8%, P = 0.049). There was no significant difference in the OS, EFS, recurrence rate or PNH clonal transformation [70].

Wei *et al*. [57] also reported the efficacy of p-ALG and CsA treatment in 69 patients with SAA. The median follow-up time was 24(4~44) months. The results showed that the ORR was 76.8% (56.5% complete and 20.3% partial). The ORR at 3, 6, 12, 18 and 24 months after ATG was 63.8%, 73.9%, 76.8%, 75.4% and 75.4%, respectively. The 2-year OS rate was 88.4%. The 2-year disease-free survival (DFS) rate was 85.5%.

A number of retrospective studies [58-70] compared the efficacy of p-ALG and r-ATG in the treatment of primary SAA. The results showed that there were no significant differences in absolute neutrophil counts and the duration of transfusion independence between p-ALG and r-ATG groups. P-ALG combined with CsA and G-CSF had a better therapeutic effect than single drug therapy, which is the same for the r-ATG group. There was no significant difference in ORR between SAA and very severe aplasia anemia (VSAA) groups treated with either p-ALG or r-ATG.

In addition, some studies have reported the efficacy of p-ALG and r-ATG as one of the conditioning drugs before transplantation. Chen *et al*. [63] analyzed and compared p-ALG with r-ATG in the pretreatment of unrelated bone marrow transplantation and unrelated peripheral hematopoietic stem cell transplantation. The OS rates of p-ALG and r-ATG groups were 47.22% and 62.45% (*P*=0.114), respectively. There was no significant difference in the incidence or severity of acute and chronic GVHD between the two groups after stratified analysis of ATG sources. Ye *et al*. [64] reported 11 patients with SAA treated with allogeneic HSCT and traditional Chinese medicine, where p-ALG was used as conditioning drug before transplantation. The 5-year OS rate was 81.8%. The incidence of grade 1-2, 3-4 adverse reactions of acute GVHD was 18.2% and 9.1%, respectively. The incidence of adverse reactions of chronic GVHD was 27.3%. Chen *et al*. [69] further evaluated the efficacy of pALG in HLA-matched sibling donor HSCT for patients with SAA. Fifty-eight patients received rATG as a part of the conditioning regimen and 55 patients received pALG. The incidence of acute and chronic GVHD was higher in pALG groups (20.7% ± 5.3% *versus* 43.4% ± 7.0%, P = .015; 20.1% ± 5.8% *versus* 46.0% ± 7.9%, P = .003). There was also no significant difference in 3-year overall survival, graft rejection or infection rate. They also compared the efficacy and safety of pALG and rATG as a part of the conditioning regimen for alternative donor allogeneic HSCT in 46 SAA patients. All 30 patients in the rATG group achieved ANC reconstitution and twenty-seven of them achieved PLT reconstitution. Five-year overall survival (OS) in pALG and rALG groups was (53.6±13.3)% and (75.1±8.2)%, respectively (P=0.190). All sixteen patients in the pALG group achieved ANC and PLT reconstitutions. There were no significant differences in the incidence of GVHD, graft rejection, bacteria, fungal or viral infection.

Tang *et al*. [65] also reported 27 patients with VSAA treated with ALG/ATG combined with traditional Chinese medicine. Sixteen patients received p-ALG, 5 received h-ATG and 6 received r-ATG treatment. The 1-year ORR of the 27 patients was 80%, including 36% basically cured, 20% remitted, and 24% significantly improved, while the other 20% cases were ineffective.

As for children patients, a study from Liu *et al*. [66] reported 70 newly diagnosed SAA children treated with pALG and CsA. The overall response rate was 57.1% at 2 years follow-up. Patients with mild disease, higher absolute reticulocyte count and platelet level displayed a higher response rate (all P < 0.001). The response rate decreased when the interval time before IST was longer than 60 days. Cui *et al*. [62] reported the efficacy of modified IST (p-ALG plus delayed CsA at day 21) in 50 SAA children. Overall response (OR) rates at 3, 6 and 12 months were 56, 64 and 62%, respectively. The 10-year overall survival (OS) rate and disease-free survival (DFS) rate were 80 and 56%, respectively. The death rate from infection within 30 days was 4%.

While for older patients, Li *et al*. [67] reported 16 cases of older SAA patients (60-79 years) treated with rATG (9 patients) or pALG (7 patients). The overall response rate was 56.3%. All 7 patients (100.0%) treated with pALG achieved a hematological response, but only two (22%) of the 9 patients treated with rATG achieved a hematological response. These data showed that pALG was superior to rATG as a first-line treatment for older SAA.

5.3. Prognostic Factors

A series of studies investigated the potential prognostic factors of AA patients. Han *et al*. [54] reported that age, sex, the severity of illness, the interval between diagnosis and treatment, and concurrent infection before treatment had no effect on ORR. Wei *et al*. [57] reported that old age, VSAA and low lymphocyte count were poor prognostic factors. Ma *et al*. [60] found that only VSAA, the interval between diagnosis and pre-treatment for more than 30 days, and no response after 6 months of treatment were poor prognostic factors. The 5-year mortality in the VSAA group was 1.5 times higher than that in the SAA group (P=0.04, 95% *CI* 1.03~5.68). The 5-year mortality was 2.6-fold higher in patients with interval time from diagnosis to treatment more than 30 days than in those with interval time less than 30 days (P=0.01, 95% *CI* 1.50~10.10), and was 2.75-fold higher in patients who did not respond at 6 months than in those who responded (P=0.15, 95% *CI* 1.01-6.04), which was similar to that reported by Liu *et al*. [58] Yang *et al*. [61] reported that patients whose course of the disease was longer than 3 months or with ANC<0.2×109/L in peripheral blood showed worse treatment responses.

Table 1. Effect of P-ALG on SAA.

References	Study Type	No. of Cases Reported	Patients	Median Age (range) Year	IST	ORR	OS	CR	Early Mortality
Han B *et al*. [49-50]	Retrospective	48	SAA	28(13-64)	p-ALG+CsA	83.3%	87.5% (1.5 year)	54.2%	10.4% (1 month)
Chen M *et al*. [51]	Retrospective	102	SAA	29(12-72)	p-ALG+CsA	71.6% (3 months); 79.4% (6months); 81.8%(5 years)	81.8% (5 years)	42.1%	16.7%
Wei J *et al*. [52]	Retrospective	69	SAA	27(14-52)	p-ALG+CsA	75.4% (24 months)	88.4% (2 years)	56.5%	10.14% (2 months)
Liu LP *et al*. [61]	Perspective	70	Newly diag-nosed SAA children	22(3-64)	p-ALG+CsA	57.1% (2 years)	100% (2 years)	40% (2 years)	0
Yang N *et al*. [62]	Retrospective	75	SAA	21(4-56)	p-ALG+CsA (43); rATG+CsA (32)	79.07%; 56.25% (6 months)	86.05%; 72.88% (5 years)	20.93%; 18.75% (6 months)	11.63%; l5.63%
Cui Q *et al*. [64]	Retrospective	50	SAA children	14(5-17)	p-ALG+CsA	56% (3months); 64% (6 months); 62%(12 months)	1080% (10 years)	Not mentioned	4% (1 month)
Chen M *et al*. [66]	Retrospective	148	SAA	30(12-72)	p-ALG+CsA (114); rATG+CsA (34)	65.8% ; 44.1%	80.5%; 74.1% (5 years)	25.4%; 23.5% (6 months)	11.4%; 11.8% (6 months)

N.: Number; ORR: Overall response rate; OS: Overall survival; CR: Complete response

5.4. Early Mortality

Han *et al*. [54] reported that the early mortality rate of 48 patients within 30 days was 10.4%. The early mortality rate of 102 expanded patients with SAA was 16.7%, and the median mortality time was 2.3 (0.2~36) months [56]. Wei *et al*. [57] reported that the overall fatality rate was 10.1% (7/69) within 60 days. Ma *et al*. [60] compared the early mortality of p-ALG group with r-ATG group and found that the mortality rates within 5 years were 15.56% and 25%, respectively, with no significant difference (P=0.302). This study indicated that the early mortality rate of SAA treated with p-ALG was about 10% ~20%. Yang *et al*. [61] reported that the early mortality rate of SAA was 11.1% (4/36). The causes of death included infection, cerebral hemorrhage, ischemic heart disease and progression to other hematopathy. The most common cause of death was an infection. Therefore, attention should be paid to the prevention of complications such as infection in the course of AA treatment to improve the efficacy and prognosis.

5.5. Clonal Evolution

In the study of 48 AA patients by Han *et al*. [54], it was not mentioned whether clonal diseases were observed or not. When the sample was enlarged and the median follow-up time was extended to 59.6 (0.2~176.8) months, clonal evolution was found in 7.8% of the patients, including 5 cases of paroxysmal nocturnal hemoglobinuria (PNH) and 2 cases of myelodysplastic syndrome (MDS) [56]. Wei [57] and Yang [61] both reported one case of a patient who developed to MDS. The clonal evolution of pALG is similar to that of rATG or hATG [71, 72]. Therefore, there is the possibility of clonal evolution in the treatment of SAA with p-ALG, which should be noticed.

5.6. Recurrence

Generally, the recurrence rate of p-ALG was low. Han [54] and Wei [57] both reported that 1 patient relapsed during the follow-up period. The recurrence rate in the study by Chen *et al*. [56] was 9.9%. Liu *et al*. [58] reported that the recurrence rate of the p-ALG group and the r-ATG group was 4.65% and 6.25%, respectively. Studies by Ma *et al*. [60] reported that the recurrence rate of the p-ALG group was 6.67% and no recurrence was observed in the r-ATG group.

5.7. Adverse Effect

Han *et al*. [54] reported that within 48 SAA patients treated with p-ALG, 25 patients (52.1%) had a fever, accompanied with rash, and recovered to normal after treatment with glucocorticoids or nonsteroidal antiinflammatory drugs. Eight patients (16.7%) had serum sickness, 29 patients (60.4%) had a liver injury, and one patient (2.1%) had hemorrhage. There were no cases of creatinine rise, anaphylactic shock and death due to p-ALG complications. In subsequent studies with enlarged sample size and prolonged follow-up time, the common adverse reactions were still allergic reactions and serum sickness reactions [22.5%(23/102)], including fever, rash and joint pain. Two patients developed autoimmune hemolytic anemia, and one of them was improved after glucocorticoid treatment, while the other died of pulmonary infection [56]. Neutropenia

often leads to infection, and thrombocytopenia can cause severe bleeding. Similarly, Wei *et al*. [57] reported that 33.3% of SAA patients had allergic reactions, 21.7% had serum sickness reactions and 27.5% had abnormal liver functions. Liu [58] and Ma *et al*. [60] reported that the adverse reactions of p-ALG and r-ATG groups were similar, and the adverse reactions could be controlled after reasonable symptomatic treatment, and most patients could tolerate the above adverse reactions.

CONCLUSION

The present studies showed that p-ALG exhibited good clinical efficacy and low incidence of adverse reactions. However, most of the above studies are single center and non-randomized clinical trials. In order to extend p-ALG to more countries or incorporate it into the standard treatment of SAA, multicenter and randomized controlled clinical trials are still needed. The best treatment of SAA and the mechanism of p-ALG also need to be further explored.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Original

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Porcine antilymphocyte globulin versus rabbit antithymocyte globulin for intensive immunosuppressive therapy of acquired aplastic anemia: A meta-analysis and systematic review

Jinsong Jia, Shasha Wang, Ying Kang, Xiujuan Liu, and Lanping Xu

Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing, China

Key words
porcine antithymocyte globulin – rabbit antithymocyte globulin – anaplastic anemia – meta-analysis

Abstract. Objective: Several studies have reported that porcine antilymphocyte globulin (pALG) has a significant effect on aplastic anemia (AA), but their conclusions are inconsistent. To objectively evaluate its efficacy and safety, a meta-analysis was conducted. Materials and methods: We systematically searched the relevant literature on pALG vs. rabbit antithymocyte globulin (rATG) as the first-line treatment in AA patients until August 31, 2022, in electronic databases: PubMed, Cochrane Library, Web of Science, etc. Two researchers independently extracted data and evaluated the quality of the study. Stata 14.0 was used for statistical analysis. Results: 50 studies were included in the analysis. The overall responses at 3, 6, and 12 months between the pALG group and rATG group were equivalent. We analyzed early mortality, total mortality, relapse rates, and 5-year survival after the administration of pALG or rATG, and there was no significant difference between the pALG and rATG groups. In our study, the incidence of infection in the pALG group was better than that in the rATG group, OR = 0.63, 95% CI (0.44 – 0.88), p = 0.008, which showed a statistically significant difference. Conclusion: The efficacy of pALG in AA patients is equivalent to that of rATG. rATG was associated with a significantly higher incidence rate of infection than pALG.

What is known about this subject

- A few previous studies have shown that there was no significant difference in survival between porcine antilymphocyte globulin (pALG) and rabbit antithymocyte globulin (rATG) in the first-line treatment of adult severe aplastic anemia (SAA) patients.
- The first-line use of pALG/rATG combined with cyclosporine A (CsA) in the treatment of children with SAA in China showed no significant difference in overall response rate (ORR), overall survival (OS), and failure-free survival (FFS) between the two groups, suggesting that there was no significant difference in the efficacy between pALG and rATG.

What this study adds

- Through this meta-analysis, it was verified that pALG and rATG have similar efficacy.
- Meanwhile, this study also has new findings that the infection incidence of pALG was slightly lower than that of rATG, which is similar to the results of horse ATG.

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Correspondence to: Jinsong Jia, Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, No. 11 Xizhimen South Street, Beijing, 100044, China, **jiajinsong@163.com**

Introduction

Background: The occurrence and development of bone marrow hematopoietic failure in patients with aplastic anemia (AA) is closely related to cellular immune disorders, especially the abnormal number and function of T cells. Therefore, abnormal immune function plays an important role in the pathogenesis of aplastic anemia [1, 2]. Severe aplastic anemia (SAA) is characterized by acute onset, rapid progression, and high mortality. Currently, intensive immunosuppressive therapy (IST) and allogeneic hematopoietic stem cell transplantation (allo-HSCT) have a significant effect on improving the prognosis of SAA. IST with antithymocyte globulin/antilymphocyte globulin (ATG/ALG) combined with cyclosporine A (CsA) can promote hematopoietic recovery in 60 – 80% of patients with SAA, which is the most direct evidence that an abnormal immune response can damage hematopoietic stem cells. However, if bone marrow failure is caused by other non-immune factors or extreme depletion of hematopoietic stem cells, IST may be ineffective. It is important to evaluate the disease characteristics and predict the curative effect before immunotherapy; moreover, abnormal cellular immune function plays an important role in the therapeutic effect of IST on newly diagnosed SAA. Allo-HSCT is recommended for young patients with SAA if an HLA-identical sibling is available. IST treatment is commonly selected as a first-line treatment for SAA patients who are not suitable candidates for allo-HSCT [3, 4]. ATG/ALG is a polyclonal immunoglobulin that is produced by animals immunized with specific antigens. Currently available ATG/ALG preparations are obtained from horses, rabbits, and pigs, and the first two are the most widely used worldwide. There are two common views on the efficacy of hATG and rATG. According to the National Institutes of Health (NIH) and EBMT Severe Aplastic Anaemia Working Party (WPSAA-EBMT), the curative effect of hATG is better than that of rATG [5, 6]. Hayakawa et al. [7] also conducted a meta-analysis on 13 clinical studies and concluded that the overall response rate (ORR) of hATG is significantly higher than that of rATG, and the early mortality of rATG is higher. However, some researchers believe that the curative effects of rATG and hATG are similar. Yang et al. [8] showed that the curative effects of rATG and hATG are similar, and there is no significant difference in ORR and overall survival (OS). In China, another antilymphocyte globulin (ALG), which is produced by pigs immunized with human thymocytes, has been used as a first-line treatment for SAA in recent years. Porcine antilymphocyte globulin (pALG) is native to China and produced by Wuhan Institute of Biological Products, National Drug Approval No. S20040023. The first-line use of pALG/rATG combined with CsA in the treatment of 189 children with SAA in the Hematological Hospital of Chinese Medical College showed no significant difference in ORR, OS, and FFS between the two groups [9], suggesting that there was no significant difference in the efficacy between pALG and rATG. However, the safety and efficacy of pALG combined with CSA for the treatment of SAA is currently lacking adequate medical-based evidence. In this study, we reviewed published studies on the use of pALG/rATG for the treatment of aplastic anemia and conducted a meta-analysis on the clinical outcomes with pALG and rATG. Through this study, it was verified that pALG and rATG have similar efficacy and safety.

Materials and methods

Literature screening and identification of relevant studies

We performed a literature search for articles published before August 31, 2022, by using the electronic databases of PubMed, Cochrane Library, Web of Science, Embase, Google Scholar, Clinical trial, CNKI (China National Knowledge Infrastructure), Wanfang data, and Chinese Biomedical Database with the following terms: “porcine antithymocyte globulin”, “pALG”, “rabbit antithymocyte globulin”, “rALG”, “aplastic anemia”, “immunosuppressive therapy” by two independent investigators (Jinsong Jia and Shasha Wang). Manual searches were also performed to identify more eligible articles in the reference lists of included articles not retrieved by the initial electronic search. We reviewed all retrieved articles by reading the titles and abstracts. Then, the full texts of the possibly

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relevant studies were examined for further suitability evaluations in our present meta-analysis. All studies in the meta-analysis were first published in the primary literature with no reproduction in other studies. All studies matching the inclusion criteria were retrieved for further examination and data extraction. All investigators received training in literature search, statistics, and evidence-based medicine.

Inclusion criteria and exclusion criteria

Studies were included if: (1) study type: RCT or cohort study; (2) study object: patients diagnosed with aplastic anemia; (3) intervention measures: porcine antilymphocyte globulin vs. rabbit antithymocyte globulin treatment; and (4) study evaluation indicators: 1) main outcome indicators, total hematological response at 3, 6, and 12 months. 2) Secondary outcome measures, 5-year survival, total mortality, early mortality, and relapse. 3) Adverse reactions, infection, allergic reaction, myelodysplastic syndrome, serum sickness, bleeding, liver function damage, renal function damage and hypertension. (5) Publication date and publication language were not restricted in our search.

The following types of articles were excluded: (1) no relevant outcome indicators were reported, or relevant data could not be obtained; (2) the same article as previous publications; (3) case studies, case series, intervention studies, qualitative studies, systematic reviews, abstracts, conference papers, meta-analyses, and repetitions of previous publications; (4) the information provided in the original literature was not enough to calculate the statistical index OR value; and (5) animal experiments.

Quality assessment and data extraction

Two investigators (Jinsong Jia and Shasha Wang) extracted data independently from the included studies using a standard protocol and data-collection form according to the above inclusion criteria and reached consensus on all items. Disagreements were resolved by discussion or consensus with a third reviewer (Lanping Xu). The data extracted from eligible studies included the first author, year of publication, participant characteristics (i.e., mean age, sample size, ethnicity), genotyping method, total numbers of cases and controls, and genotype frequencies of cases and controls.

The quality of the included studies was assessed by two independent reviewers (Ying Kang and Xiujuan Liu) according to the Newcastle-Ottawa Quality Assessment Scale (NOS) for case-control studies. The evaluation contents of the scale included the selection of patients, the comparability and outcome of the study, and the score distribution ranging from 0 to 9. Research with a score greater than or equal to 6 was identified as high-quality literature and was included in the study. The quality of the literature was independently evaluated by two researchers. If the evaluation results were inconsistent, they were upon decided by discussion and negotiation.

Intervention and outcome

We obtained data on study design, control measures, patient number, types of ATG/ALG, dosage of ATG/ALG, early mortality at 3 months, and overall response at 6 months. We extracted data on the year of ATG/ALG administration, censored data, and patient baseline characteristics for each group when available. The baseline patient characteristics included age, sex, etiology (idiopathic or hepatitis associated), disease severity, interval between diagnosis and treatment, use of CsA, use of granulocyte colony-stimulating factor (G-CSF), presence of paroxysmal nocturnal hemoglobinuria clone, and pretreatment hematologic parameters such as absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute reticulocyte count (ARC), and platelet count. When the exact value for the early mortality rate was not presented, it was estimated from Kaplan-Meier survival curves given in the publication.

The clinical outcomes of pALG and rATG were compared. The primary outcome measure was the early mortality rate at 3 months after treatment with ATG/ALG. The secondary outcome measure was the overall response at 6 months. The definition of overall response was almost uniformly defined as

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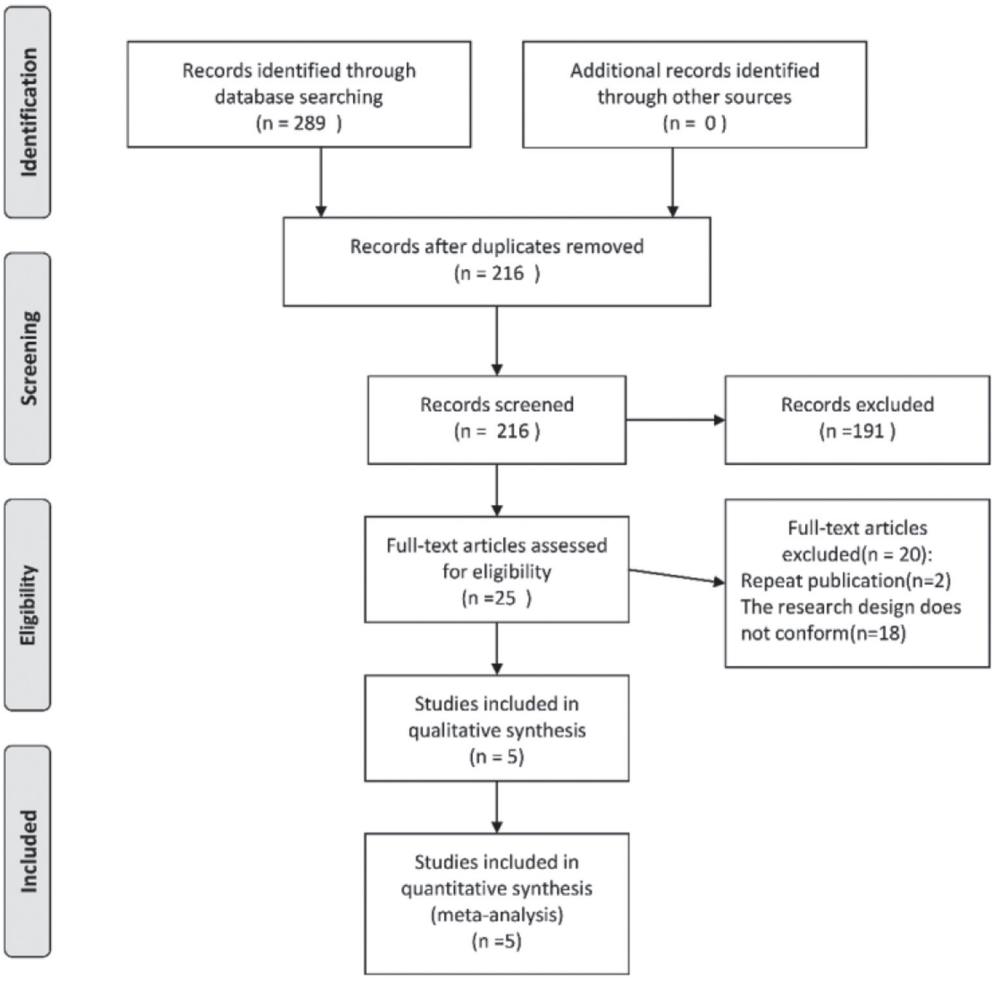

Figure 1. Flow diagram of study selection. Five publications were deemed eligible.

blood count recovery not meeting the severe aplastic anemia criteria and transfusion independency, except for one study in which only transfusion independency was needed. However, removing this study from the sensitivity analysis did not affect the conclusion.

Data synthesis and statistical analysis

All statistical analyses were performed with Revman 5.3 and Stata 14.0 software (STATA Corporation, College Station, TX, USA). If the data of two categories were compared, the statistical results were expressed by odds ratio (OR) and 95% confidence interval (CI). The heterogeneity of the study was tested by χ^2-test (the test level was $\alpha = 0.1$) and I2 statistics. If $p < 0.1$ or I2 > 50%, which indicates that there was heterogeneity among the research results, the random effect model was used for the combined analysis. Otherwise, the fixed effect model was used. The stability and reliability of the meta-analysis was evaluated by sensitivity analysis. Egger's and Begg's tests were used to evaluate potential publication bias. All p-values are two-sided, and $p < 0.05$ served as the threshold of statistical significance.

Results

Search results

After the first search in the PubMed, Cochrane Library, Science, Embase, Google Scholar, Clinical trial, CNKI, CBM, and WanFang databases, 289 articles were retrieved. No additional studies were included after further manual search of reference lists. After importing into Endnote, 73 duplicate

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Table 1. Characteristics and outcomes of eligible studies.

Author (publication year)	Region	Study design	Centers, number	Treatment year	Group, number (pig/rabbit)	Age, years (pig/rabbit)	Follow-up, months (pig/rabbit)	Definition of early mortality
Kong et al. (2019) [11]	China	Retrospective	1	2011 – 2017	63/103	7 (1 – 5)/7 (2 – 11)[a]	38 (16 – 81)/25 (16 – 79)[a], months	NA
Ma et al. (2016) [13]	China	Retrospective	1	2004 – 2013	43/32	21 (4 – 56)/24.5 (7 – 64)[a]	38 (16 – 81)/25 (16 – 80)[a], months	Early 3 months
Zhu et al. (2020) [9]	China	Retrospective	1	2014 – 2018	142/47	12 (4 – 16)/11 (5 – 16)[a]	1,370 (86 – 2,025)/1,416 (22 – 2,027)[a], days	Early 3 months
Liu et al. (2015) [12]	China	Retrospective	1	2005 – 2013	43/32	22 (3 – 64)/23 (3 – 70)[a]	29 (5 – 103)/28 (3 – 100)[a], months	Early 6 months
Chen et al. (2018) [10]	China	Retrospective	1	1996 – 2015	114/34	30 (12 – 72)/35.5 (14 – 76)[a]	70.3 (6.1 – 138.5)/29.85 (9.1 – 158.6)[a], months	Early 3 months

NA = data not available; [a]medians in each study.

references were deleted. Full texts of the remaining 25 records were obtained after exclusion for improper titles and/or abstracts. 20 studies were excluded (repeat publication (n = 2) and ineligible study design (n = 18)). After exclusion, 5 studies [10, 11, 12, 13, 14] involving a total of 653 disease cases met the inclusion criteria and were subjected to further examination (Figure 1).

Characteristics of the included studies and quantitative synthesis

The characteristics of the studies are summarized in Tables 1 and 2. All studies were retrospective cohort studies and were conducted in China from 1996 to 2018 [10, 11, 12, 13, 14]. All studies were published between 2015 and 2020. The sample size varied between 75 and 189. The median or mean age of the patients ranged from 7 to 35.5. CsA was used for all patients in all studies. The ALG dose was 20 – 30 mg/kg/day for 5 days, and the rATG dose was 2.5 – 5 mg/kg/day for 5 days. The results of the literature quality evaluation are shown in Table 3. The scores are all 7 – 8, which indicate high-quality literature.

Meta-analysis results

Overall response

We analyzed the total hematological response at 3, 6, and 12 months, and the heterogeneity results (I2 fluctuated between 0 and 21%, $p > 0.1$). The fixed effect model was used for meta-analysis. The results showed that the total hematological responses of the pALG group and rATG group at 3, 6, and 12 months were equivalent, with OR = 1.36, 95% CI (0.96 – 1.94), $p = 0.08$; OR = 1.40, 95% CI (0.92 – 2.12), $p = 0.12$; and OR = 1.29, 95% CI (0.79 – 2.11), $p = 0.32$, respectively (Figure 2).

5-year survival

Three studies [10, 13, 14] evaluated the 5-year survival rate, and the heterogeneity test results were I2 = 0%, $p = 0.427$. The fixed effect model was used for meta-analysis. The results showed that the 5-year

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Table 2. Study characteristics.

Author (publication year)	pALG dose	rATG dose	CSA dose	VSAA (%) (pig/rabbit)	NEUT (pig/rabbit)	PLT (pig/rabbit)	RET (pig/rabbit)
Kong et al. (2019) [11]	25 mg/kg/day for 5 days (Wuhan Institute of Biological Products Co. Ltd., Wuhan, China)	3.75 mg/kg/day for 5 days (IMTIX SANGSTAT,France)	3 – 5 mg/kg per day	29 (46.00)/ 47 (45.60)	0.57 (0.21 – 1.03)/ 0.67 (0.27 – 1.04)[a]	23 (11 – 29)/ 20 (8 – 25)[a]	NA
Ma et al. (2016) [13]	25 mg/kg/day for 5 days (Wuhan Institute of Biological Products Co. Ltd., Wuhan, China)	2.5 mg/kg/day for 5 days (thymoglobulin; Genzyme Corp., Cambridge, MA, USA))	3 – 5 mg/kg per day (Novartis International AG, Basel, Switzerland)	14 (31.11)/ 13 (40.63)	0.24 (0.02 – 1.03)/ 0.37 (0 – 1.0)[a]	13 (2 – 20)/ 11.5 (6 – 20)[a]	0.12 (0.03 – 1.73)/ 0.16 (0.07 – 1.52)[a]
Zhu et al. (2020) [9]	20 mg/kg/day for 5 days (Wuhan Institute of Biological Products Co. Ltd., Wuhan, China)	3 mg/kg/day for 5 days (thymoglobulin; Genzyme Corp., Cambridge, MA, USA))	3 – 5 mg/kg per day	48 (34)/ 16 (34)	0.63 ± 0.7/ 0.70 ± 0.95	24.0 ± 17.6/ 25.8 ± 18.8	25.6 ± 22.9/ 21.9 ± 17.7
Liu et al. (2015) [12]	30 mg/kg/day for 5 days	3.75 mg/kg/day for 5 days (thymoglobulin; Genzyme Corp., Cambridge, MA, USA))	3 – 5 mg/kg per day	15 (34.88)/ 11 (34.37)	0.35 ± 0.04/ 0.33 ± 0.03	8.0 ± 1.0/ 7.0 ± 0.9	10.4 ± 0.9/ 11.2 ± 1.1
Chen et al. (2018) [10]	20 – 30 mg/kg/day for 5 days (Wuhan Institute of Biological Products Co. Ltd., Wuhan, China)	3 – 5 mg/kg/day for 5 days (Genzyme Polyclonals S.A.S, Lyon, France))	3 – 5 mg/kg per day	37 (32.5)/ 17 (50.0)	0.46 ± 0.46/ 0.26 ± 0.24	12.43 ± 8.27/ 11.15 ± 9.16	19.66 ± 17.95/ 14.35 ± 11.42

pALG = porcine anti-lymphocyte immunoglobulin; rATG = rabbit anti-thymocyte immunoglobulin; VSAA = very severe aplastic anemia; NEUT = neutrophil; RET = reticulocyte; PLT = platelet; NA = data not available; [a]medians in each study.

survival of the pALG group and rATG group was similar, OR = 1.27, 95% CI (0.72 – 2.25), p = 0.409, as shown in Table 3.

Total mortality

Five studies [10, 11, 12, 13, 14] evaluated the total mortality rate. The heterogeneity test results were I2 = 0%, p = 0.666. The fixed effect model was used for meta-analysis. The results showed that the total mortality rates of the pALG group and rATG group were similar, OR = 0.78, 95% CI (0.43 – 1.39), p = 0.399, as shown in Table 3.

Early mortality

Four studies [10, 12, 13, 14] evaluated the early mortality rate. The heterogeneity test results were I2 = 0%, p = 0.914. The fixed effect model was used for meta-analysis. There was no significant difference in early mortality between the pALG and rATG groups, OR = 0.98, 95% CI (0.43 – 2.25), p = 0.399, as shown in Table 3.

Relapse

Four studies [10, 12 , 13, 14] evaluated the relapse rate. The heterogeneity test results were I2 = 0%, p = 0.714. The fixed effect model was used for meta-analysis. The results showed that the relapse rates of the pALG group and rATG group were similar, OR = 1.75, 95% CI (0.66 – 4.63), p = 0.257, as shown in Table 3.

Adverse responses

Table 4 shows that there is no statistically significant difference between the pALG group and rATG group in the incidence rate of adverse reactions, such as allergic reaction, myelodysplastic syndrome, serum sickness, bleeding, liver function damage, renal function damage, brain function damage, and hypertension, and the effect volumes are OR = 0.61, 95% CI (0.37 – 1.03), p = 0.062; OR = 0.59, 95% CI (0.12 – 2.91), p = 0.515; OR = 1.02, 95% CI (0.70 – 1.48), p = 0.92; OR = 1.13, 95% CI (0.49 – 2.59), p = 0.782; OR = 0.91, 95% CI (0.49 – 1.69), p = 0.759;

Table 3. Quality of literature included in the meta-analysis.

Author (publication year)	Selection (score)	Comparability (Score)	Measure of the exposure factor (score)	Total (score)
Kong et al. (2019) [11]	4	1	3	8
Ma et al. (2016) [13]	4	1	2	7
Zhu et al. (2020) [9]	4	2	2	8
Liu et al. (2015) [12]	4	1	3	8
Chen et al. (2018) [10]	4	1	2	7

OR = 1.20, 95% CI (0.38 – 3.71), p = 0.758; OR = 1.74, 95% CI (0.61 – 5.02), p = 0.302; respectively. However, the incidence of infection in the pALG group was lower than that in the rATG group, OR = 0.63, 95% CI (0.44 – 0.88), p = 0.008, which showed a statistically significant difference.

Sensitivity analysis

The stability and reliability of the meta-analysis were evaluated by sensitivity analysis. The outcome measures were stability and reliability. The sensitivity analysis is shown in Supplemental Figure 3 – 17, which indicates that the meta-analysis results are stable.

Publication bias

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of the included studies. The results are shown in Table 4 and Supplemental Figures 18 – 32. Except for the publication bias of allergic reaction, there was no publication bias in other outcome indicators.

Discussion

Aplastic anemia is an immune-mediated bone marrow disorder that is associated with bone marrow cell destruction and peripheral blood pancytopenia [1, 2]. IST is the first-line treatment for patients who are unsuitable for allo-HSCT. The combined use of anti-human thymocyte immunoglobulin (ATG) and CsA is now recognized as the standard intensive IST, which markedly improves the prognosis of patients with SAA. hATG is still unavailable in the Chinese market, and only rATG and pALG are available. Thus far, a few reports have retrospectively compared the efficacy of pALG and rATG. Recent research shows that there is no significant difference between the therapeutic effects of pALG and rATG. In the current study, the

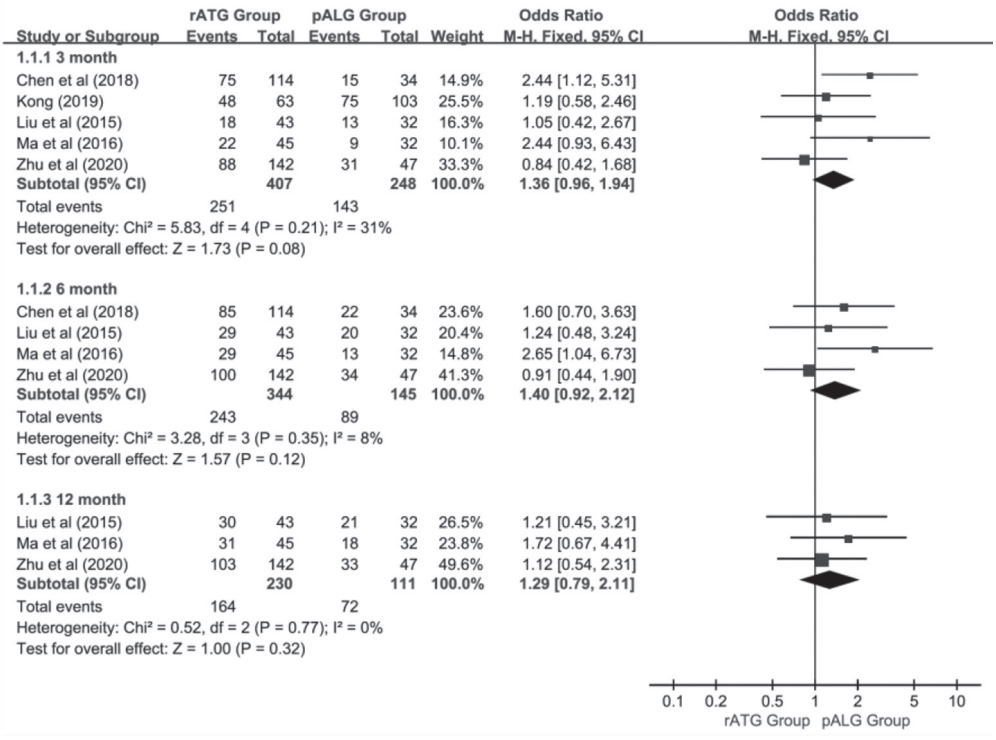

Figure 2. Forest plots for overall response rate at 3, 6, and 12 months with rATG (thymoglobulin) vs. pALG (lymphoglobulin). The results are shown in forest plots. The effect size and 95% confidence interval for each study are represented by a square and bar, respectively. Integrated risk ratios are represented by diamonds.

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Table 4. Results of meta-analysis of each outcome.

Factors	Studies (n)	Analytical model	OR (95%CI)	p-value	Heterogeneity		Publication bias	
					I^2 (%)	P_h	Begg's P	Egger's P
Overall response								
3 months	5	FEM	1.36 (0.96 – 1.94)	0.080	21	0.210	0.327	0.496
6 months	4	FEM	1.40 (0.92 – 2.12)	0.120	8	0.120	0.497	0.390
12 months	3	FEM	1.29 (0.79 – 2.11)	0.320	0	0.770	0.602	0.680
5-year survival	3	FEM	1.27 (0.72 – 2.25)	0.409	0	0.424	0.602	0.528
Total mortality	5	FEM	0.78 (0.43 – 1.39)	0.399	0	0.666	0.624	0.284
Early mortality	4	FEM	0.98 (0.43 – 2.25)	0.961	0	0.914	0.497	0.635
Relapse	4	FEM	1.75 (0.66 – 4.63)	0.257	0	0.714	0.174	0.566
Adverse responses								
Infection	5	FEM	0.63 (0.44 – 0.88)	0.008	0	0.603	0.050	0.071
Allergic reaction	3	FEM	0.61 (0.37 – 1.03)	0.062	0	0.485	0.117	0.013
Myelodysplastic syndrome	3	FEM	0.59 (0.12 – 2.91)	0.515	0	0.619	0.117	0.402
Serum sickness	4	FEM	1.02 (0.70 – 1.48)	0.920	0	0.569	0.327	0.120
Bleeding	3	FEM	1.13 (0.49 – 2.59)	0.782	0	0.968	0.117	0.230
Liver function damage	3	FEM	0.91 (0.49 – 1.69)	0.759	0	0.519	0.117	0.363
Renal function damage	3	FEM	1.20 (0.38 – 3.71)	0.758	0	0.386	0.117	0.296
Hypertension	2	FEM	1.74 (0.61 – 5.02)	0.302	0	0.968	0.317	--

p-values were obtained by using the "metan" program in Revman5.3. p-value,0.05 was considered as statistically significant; FEM = fixed-effects model; OR = odds ratio; CI = confidence interval.

results showed that the overall responses at 3, 6, and 12 months between the pALG group and rATG group were equivalent. We analyzed early mortality, total mortality, relapse rates, and 5-year survival after the administration of ATG/ALG, and there was no significant difference between the pALG and rATG groups. The results regarding the overall response rate in the present study are consistent with those of retrospective cohort studies conducted by other centers, in which pALG treatment showed the same outcomes as rATG treatment with respect to both overall survival and the response rate [9, 10, 11, 12, 13]. Zhu et al. [9] conducted a single-center, non-randomized, retrospective cohort study to evaluate the efficacy of first-line use of pALG or rATG combined with CsA in the treatment of 189 children with SAA. The results showed that there was no significant difference in the total effective rate of pALG and rATG at 3 months, 6 months, 12 months, and 24 months (3 months: 61.9 vs. 67.4%, p = 0.5; 6 months: 70.9 vs. 73.9%, p = 0.69; 12 months: 77.3 vs. 73.3%, p = 0.58; 24 months: 81.6 vs. 78.6%, p = 0.59). Chen et al. [10] retrospectively analyzed 148 patients with SAA who received pALG or rATG combined with CSA. The results showed that the overall response rates of the pALG and rATG groups at 6 months after treatment were 74.6% and 64.7%, respectively (p = 0.361). There was no significant difference in overall survival and failure-free survival between the two groups. These results show that there was no significant difference in the OR or OS of pALG compared with rATG.

SAA needs to be given appropriate IST as soon as possible, but the intensity of treatment is not always better. The main therapeutic mechanism of ATG is the clearance of T lymphocytes in the circulating pool by antibody-dependent cell-mediated cytotoxicity (ADCC). Marsh et al. [6] found that rATG and hATG had similar effects in scavenging $CD8^+$ T cells, but rATG had a stronger effect in killing $CD4^+$ T cells, especially in reducing $CD4^+$ regulatory T cells (Tregs). Therefore, lymphocyte depletion was more severe and persistent after rATG treatment, which is one of the reasons why the time from treatment to hematological remission of rATG is longer than that of hATG [7, 8]. In addition, long-term lymphocyte deficiency will affect the recovery of bone marrow hematopoietic function and increase the incidence of infection. Many studies have reported that rATG has deep immunosuppression and a high incidence of infection [5, 6, 7, 8, 15, 16]. Furthermore, longer-lasting immunosuppression and no response to IST in patients receiving thymoglobulin could increase the likelihood of infection and early mortality.

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In our study, the incidence of infection in the pALG group was better than that in the rATG group, OR = 0.63, 95% CI (0.44 – 0.88), p = 0.008, which showed a statistically significant difference. The difference in the incidence of infection between hALG and rATG may be attributable to the differences in their immunosuppressive effects. rATG exerts a stronger immunosuppressive effect, leading to sustained suppression of lymphocytes [15, 17]. Chen et al. [10] found that the rATG group had a significantly lower minimum number of lymphocytes than the pALG group; the lymphocytes of the rATG group remained significantly lower after 1, 3, and 6 months of treatment and returned to the level of the pALG group after 12 months of treatment. These findings indicate more clearance of peripheral blood lymphocytes by rATG than by pALG, with a longer duration of persistence. Therefore, similar to the comparative study of rATG and hATG, the stronger immunosuppressive effect did not improve the efficacy of treating SAA [7]. rATG has more severe and persistent lymphocytopenia and a stronger immunosuppressive effect than pALG and cannot improve the therapeutic effect of SAA. This result encourages further studies on the different effects of pALG and rATG on the immune system. Although the pathophysiology of aplastic anemia is not fully understood, a decrease in and functional abnormality of $CD4^+CD25^+FOXP3^+$ Tregs has been noted in aplastic anemia patients [18, 19, 20]. The frequency of Tregs in severe aplastic anemia is lower than that in moderate aplastic anemia and is increased after IST [21]. Tregs promote normal hematopoiesis and hematopoiesis after HSCT under stressful conditions [22, 23]. Although the frequency of Tregs in lymphocytes was increased by rabbit ATG [17], the absolute number of Tregs was decreased due to a profound decrease in the absolute lymphocyte count in vivo, which may result in a lower response rate in the rATG group.

In conclusion, studies concerning the efficacy of hATG and rATG found that pALG is equivalent to rATG. This study provides information about the options for first-line drugs for intensive IST treatment of SAA patients in China who are not transplant candidates. The difference in the immunosuppressive effect of the two drugs on SAA will be determined in further studies.

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Authors' contributions

Jinsong Jia and Lanping Xu designed the study. Shasha Wang, Ying Kang, Xiujuan Liu, and Jinsong Jia performed a publication search for articles. Jinsong Jia and Shasha Wang extracted and analyzed the data. Jinsong Jia wrote the manuscript; all authors reviewed the manuscript and approved the final version for submission.

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Conflict of interest

The authors declare that they have no conflict of interest.

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