children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013; **14**: 199-209.

24) Bostrom BC, Sensel MR, Sather HN, et al. Children's Cancer Group. Dexamethasone versus prednisone and daily oral versus weekly mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood. 2003; **101**: 3809-3817.
25) Mitchell CD, Richards SM, Kinsey SE, et al. Medical Research Council Childhood Leukaemia Working Party. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. Br J Haematol. 2005; **129**: 734-745.
26) Larsen EC, Salzer WL, Devidas JB, et al. Comparison of high-dose methotrexate (HD-MTX) with Capizzi methotrexate plus asparaginase (C-MTX/ASNase) in children and young adults with high-risk acute lymphoblastic leukemia (HR-ALL): a report from the Children's Oncology Group Study AALL0232 [abstract]. J Clin Oncol. 2011; **29**: Abstract3.
27) Vrooman LM, Stevenson KE, Supko JG, et al. Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study—Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol. 2013; **31**: 1202-1210.
28) Möricke A, Reiter A, Zimmermann M, et al. German-Austrian-Swiss ALL-BFM Study Group. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood. 2008; **111**: 4477-4489.
29) Schrappe M, Reiter A, Zimmermann M, et al. Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Münster. Leukemia. 2000; **14**: 2205-2222.
30) Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Faber Consortium Protocol 91-01. Blood. 2001; **97**: 1211-1218.
31) Pui CH, Howard SC. Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol. 2008; **9**: 257-268.
32) Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009; **360**: 2730-2741.
33) Childhood ALL Collaborative Group. Duration and intensity of maintenance chemotherapy in acute lymphoblastic leukaemia: overview of 42 trials involving 12 000 randomised children. Lancet. 1996; **347**: 1783-1788.
34) Conter V, Valsecchi MG, Silvestri D, et al. Pulses of vincristine and dexamethasone in addition to intensive chemotherapy for children with intermediate-risk acute lymphoblastic leukaemia: a multicentre randomised trial. Lancet. 2007; **369**: 123-131.
35) De Moerloose B, Suciu S, Bertrand Y, et al. Children's Leukemia Group of the European Organisation for Research and Treatment of Cancer (EORTC). Improved outcome with pulses of vincristine and corticosteroids in continuation therapy of children with average risk acute lymphoblastic leukemia (ALL) and lymphoblastic non-Hodgkin lymphoma (NHL): report of the EORTC randomized phase 3 trial 58951. Blood. 2010; **116**: 36-44.
36) Toyoda Y, Manabe A, Tsuchida M, et al. Six months of maintenance chemotherapy after intensified treatment for acute lymphoblastic leukemia of childhood. J Clin Oncol. 2000; **18**: 1508-1516.
37) Schultz KR, Bowman WP, Aledo A, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol. 2009; **27**: 5175-5181.
38) Schultz KR, Carroll A, Heerema NA, et al. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children's Oncology Group Study AALL0031. Leukemia. Prepublished on January 14, 2014, as doi: 10.1038/leu.2014.30.
39) Biondi A, Schrappe M, De Lorenzo P, et al. Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukaemia (EsPhALL): a randomised, open-label, intergroup study. Lancet Oncol. 2012; **13**: 936-945.
40) Weston BW, Hayden MA, Roberts KG, et al. Tyrosine kinase inhibitor therapy induces remission in a patient with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. J Clin Oncol. 2013; **31**: e413-e416.

第75回日本血液学会学術集会
シンポジウム9
Late adverse effects of HSCT

造血幹細胞移植後の生ワクチン接種

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Key words : Hematopoietic stem cell transplant, Live vaccines

1. 緒　言

造血幹細胞移植患者は，肺炎球菌感染症，インフルエンザ桿菌感染症，麻疹，水痘，インフルエンザといったvaccine-preventable disease（VPD）の罹患高リスクであることが報告されている[1~5]。一方で造血幹細胞移植患者のVPDは報告される症例が限られており，VPDへの抗体価が低下していることに対する臨床的な影響は必ずしも実感しにくい。

日本においては麻疹，風疹ともにまだ地域的な流行がみられている[6~8]。2013年には風疹の流行がみられ，それに伴い2012年には4例であった先天性風疹症候群も2013年には32例と増加した。2014年には麻疹の流行がみられており，2013年のB3型の報告は26件/年であったのに対し，2014年1月にはすでに52例と増加している。造血幹細胞移植後に麻疹で死亡する症例があり[9]，患者本人をVPDから守るだけでなく，今後移植経験者が増えていくなかでその家族を守るためにも重要である。水痘・帯状疱疹ウイルス感染症は現在でも散発的に流行が見られており，造血幹細胞移植後の患者では重症化して致死的となることも少なくない[4]。

しかし，造血幹細胞移植後の患者のフォローアップにおけるワクチン接種の重要性に比して，その理解は十分に広がっているとは言い難い。本稿では，特に移植後の生ワクチン接種に重点を置いて概説する。

2. 造血幹細胞移植後の獲得免疫能の低下

VPDに対する移植前からの抗体価は，自家移植，同種移植ともに造血幹細胞移植後1~10年で消失し[10,11]，小児を含めた若年者，予防接種者（非麻疹罹患者），急性graft versus host disease（GVHD）Grade II~IVの既往のある者では特に麻疹抗体価が陰転化しやすいことが報告されている[12]。当センターの検討でも，移植後患者ではワクチン再接種あるいは麻疹患者との接触がなかった12例中12例全例で麻疹抗体価がNT 4倍以下となっており，再接種が望まれた[13]。2002年6月に行われた日本小児血液学会・造血幹細胞移植委員会による骨髄移植推進財団認定施設に対するアンケート調査では170/174施設でアンケート回収があり，21施設で合計37名の麻疹発症があり，そのうち8.1%にあたる3名（小児2名，成人1名）が死亡している[9]。このようにワクチンで予防できる疾患で患者を亡くさないためにも，造血細胞移植経験者における長期フォローアップの一環として，移植後のワクチン再接種が望ましい。

3. 造血幹細胞移植後の免疫再構築

造血幹細胞移植後には免疫学的発生に沿って自然免疫系が先に回復し，その後，獲得免疫系が回復していく[14]。自然免疫系の骨髄球系細胞とNK細胞はいずれも移植後6か月までの早期に回復する。一方で，獲得免疫系であるリンパ球の免疫学的回復は，新生児期からのリンパ球発達過程に沿って回復し，新たな免疫を獲得するT細胞レパートリーの再構築は胸腺機能が回復する移植後6か月以降に開始し，細胞性免疫が完全に回復するには年単位の時間を要する[15]。

B細胞数は移植後1~3か月はほぼないに等しく，3~12か月かけて増加し正常化していく。T細胞数も移植後1~3か月は少なく，その回復速度は年齢に左右され，それは胸腺機能の回復速度によると考えられている[14]。慢性GVHDのない18歳未満の患者ではCD4+T細胞が200/μlを超えるのは6~9か月なのに対し，成人で特にGVHDを有する患者では2年以上を要する[1]。世界各国のガイドラインで生ワクチン接種が移植後24か月以降に設定されているのはこのためと思われる。成

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人で移植後1年以内に血中に循環しているT細胞の多くはメモリー/エフェクターT細胞で，それらはおそらく移植片に混入したドナーリンパ球であり，ドナーが遭遇してきた抗原に対し反応する。しかしそれらはいずれ消失する。そして移植された造血幹細胞由来の，新たな抗原に対し反応するナイーブT細胞は移植後6～12か月して産生されてくる[1]。

GVHDあるいはその治療はワクチンへのT細胞や抗体の反応を妨げる。一方で慢性GVHDを伴う患者はむしろ免疫不全の状態にあるため，ワクチンによる保護が必要である。海外のガイドラインでは生ワクチンを除いて慢性GVHDのある患者へのワクチン接種の延期は推奨しておらず[1]，日本においても慢性GVHDの増悪がなければ不活化ワクチンの接種は制限していない[16]。一方で生ワクチン接種に関しては，免疫抑制剤服用中の移植後患者においても安全に接種可能であったという報告はあるものの[17,18]，その症例数はまだ少なく，慢性GVHDがあり免疫抑制剤服用中の移植後患者に対しては一般的には推奨されていない[1,16]。

4. 予防接種の時期とスケジュール

造血幹細胞移植経験者への予防接種ガイドラインはEuropean Group of Blood and Marrow Transplantation（EBMT）とCenters for Disease Control（CDC）とで統一されたものが2009年に発表された[1]。日本造血細胞移植学会が発行した予防接種ガイドラインの接種スケジュールと合わせてTable 1, 2に示す。生ワクチン接種に関して，海外のガイドラインが造血細胞移植学会の予防接種ガイドラインと違う点は，海外ではMeasles, Mumps, and Rubella vaccine（MMR）が使用されており小児では2回接種が推奨されていることと，水痘・帯状疱疹ワクチンは推奨されていないことがあげられる。時期に関してはいずれも移植後24か月以降で共通しており，海外のガイドラインでは加えて活動性のあるGVHDがなく免疫抑制剤が中止となっていることが条件として記載がある。造血細胞移植学会のガイドラインではさらに免疫学的回復が得られた時期とされているが，免疫学的回復の具体的な指標は記されていない。日本小児白血病リンパ腫研究グループ（Japanese Pediatric Leukemia/Lymphoma Study Group, JPLSG）長期フォローアップ委員会による長期フォローアップガイドライン[19]では，PHA刺激によるリンパ球幼若化反応が正常化，CD4細胞数500/μ以上を提案している。なお，Memorial Sloan Kettering Cancer Centerにおいては生ワクチン接種の基準のひとつとしてCD4数200/μ以上を採用しているが，接種に関する重篤な有害事象は生じていない[20]。

5. 生ワクチン接種の安全性と有効性

小児と若年成人において，移植後24か月が経過し，GVHDや免疫抑制剤の使用がない造血幹細胞移植経験者に対するMMRワクチンの安全性と有効性が報告されており，ワクチン接種による抗体価陽転率は麻疹，風疹，ムンプスでそれぞれ77%，64%，75%であった[21]。一方でSpoulouらは小児を対象として有効性の検討を行っているが，MMRワクチン接種12か月後の抗体価陽性率は麻疹，風疹，ムンプスで23.3%，90%，36.6%と大きく異なっている[22]。各研究により結果に差がみられている理由は明確ではないが，国や年代が異なることによる環境の違い，また抗体価の測定法の違いが一因である可能性は否定できない。製剤の違いもあるため，海外での安全性と有効性に関するエビデンスを必ずしもそのまま日本にあてはめることはできない。そのため当センターでの経験から同種造血幹細胞移植後の生ワクチン接種に対する安全性と有効性について検討を行ったのでその結果を次項に記す（投稿中）。

最近，ワクチン接種による抗体価がどのように推移していくかについての研究報告もみられてきており，世界的にも造血幹細胞移植後のVPD予防がいかに注目されつつあるかがうかがえる。小児同種造血幹細胞移植経験者210名のワクチン接種後の抗体価を前向きに追跡した結果，5年以上の抗体価陽性の持続は破傷風，風疹，ポリオに関しては95.7%，92.3%，97.9%とほとんどの症例で確認されたのに対し，百日咳，麻疹，ムンプス，B型肝炎では25.0%，66.7%，61.5%，72.9%と芳しくなく，ワクチンによって異なる結果であった[23]。観察期間中に抗体価が陰性化する症例も少なくなかった。Vaccine failureのリスク因子としては，ワクチン接種時の年齢が高いこと，CD3，CD4あるいはCD19細胞数が少ないこと，血清IgM値が高いこと，サイトメガロウイルス抗体価陽性であること，ワクチン接種時にその抗体価が陰性であること，GVHDの既往があること，TBIを含む前処置であることがあげられた。これらの結果から，vaccine failureの高リスク群では長期の抗体価フォローが必要であり，抗体価陰性者は再接種をすべきであるとしている。

6. 当センターでの経験から

2000年1月から2013年9月までに当センターで同種造血幹細胞移植を施行し1年以上生存している患児のうち，生ワクチンの接種を行い，その後抗体価を測定しえた44名を対象にし，接種後の抗体価を後方視的に解析した。当センターでの生ワクチン接種開始の基準は当院感染免疫科医師と共同で検討し，2009年4月以降は

Table 1 Vaccinations recommended by The Center for International Blood and Marrow Transplantation Research[1)]

Vaccine	Evidence level	Time post-HCT to initiate vaccine	No. of doses
recommended			
Pneumococcal conjugate (PCV)	B I	3–6 months	3–4[a]
Haemophilus influenzae conjugate	B II	6–12 months	3
Tetanus, diphtheria, acellular pertussis	Tetanus, diphtheria: B II Pertussis: C III	6–12 months	3
Inactivated polio	B II	6–12 months	3
Meningococcal conjugate	Follow country recommendations for general population: B II	6–12 months	1
Recombinant hepatitis B	Follow country recommendations for general population: B II	6–12 months	3
Inactivated influenza	A II	4–6 months	1–2[b]
Measles-Mumps-Rubella (live)	Measles: All children and seronegative adults, B II Mumps: C III Rubella: B III E III (<24 months post HCT, active GVHD, on immunosuppression)	24 months	1–2[c]
optional			
Varicella (Varivax, live)	C III (>24 months, without active GVHD or on immunosuppression) E III (<24 months post HCT, active GVHD or on immunosuppression)		
Japanese B encephalitis	According to local policy when residing in or travelling to endemic areas: C III	no data exist	
Human papillomavirus	Follow country recommendations for general population: C III	no data exist	
not recommended			
Zoster vaccine (Zostavax, live)	E III (No data regarding safety among HCT recipients)		
Rotavirus	E III (Must be given before 12 weeks of age to be safe)		

[a] Following the primary series of three PCV doses, a dose of the 23-valent polysaccharide pneumococcal vaccine (PPSV23) to broaden the immune response might be given (B II). For patients with chronic GVHD who are likely to respond poorly to PPSV23, a fourth dose of the PCV should be considered instead of PPSV23 (C III).

[b] For children <9 years of age, two doses are recommended yearly between transplantation and 9 years of age.

[c] In children, two doses are favored.

Table 3を原則としている。2009年3月以前は主治医ごとに接種開始が判断されており，統一した開始基準は存在していなかったが，移植後12か月を経過し，免疫抑制剤の使用がないことを原則としている。しかし，周囲の流行状況によっては移植後12か月未満であっても接種を行った。抗体価の測定はEIA法（IgG）で行い，陽性基準は日本造血細胞移植学会の「造血細胞移植ガイドライン　予防接種」を参考に，麻疹，風疹は8.0以上，水痘，ムンプスは6.0以上とした。

患者背景は，男児27名，女児17名で移植時年齢は中央値5.6歳（0.5歳から16.4歳）であり，ワクチン接種の開始時期が不明の3名を除いた41名において，移植から予防接種開始までの期間は，中央値20.0か月（4.8か月から111.3か月）で予防接種開始からの観察期間は中央値75.2か月（4.8か月から145.1か月）であった。63.4%の症例が24か月未満での接種開始であったにもかかわらず，治療を必要とする有害事象はなく，ワクチン接種後の抗体陽性率は麻疹62.8%，風疹67.6%，水痘58.6%，ムンプス12.5%であった。日本における生ワクチン接種も安全であり，その有効性もムンプスを除き既報と大差ない結果と思われた[20]。しかし，vaccine failureが少なくないのも事実であり，抗体価を測定しながら追加接種も必要であると考えている。予防接種後の抗体陰性群と抗体陽性群で移植時年齢，予防接種開始の時期，予防接種開始時のリンパ球数，血清IgG値，背景疾患，前処置，ドナー情報，急性GVHD，慢性GVHDの有無で比較をしたが，複数のワクチンで再現される有意差のある因子は同定できなかった。しかしムンプスを

Table 2 Vaccinations recommended by The Japan Society for Hematopoietic Cell Transplantation[16]

Vaccine	Time post-HCT to initiate vaccine	No. of doses
23-valent pneumococcal	6–12 months without active cGVHD	1[a]
Haemophilus influenaze conjugate	6–12 months without active cGVHD	3
Tetanus, diphtheria, acellurlar pertussis	6–12 months without active cGVHD	3
Recombinant hepatitis B	6–12 months without active cGVHD	3[b]
Inactivated influenza	6–12 months without active cGVHD	1–2[c]
Measles-rubella	24 months without active GVHD or on immunosuppression	1
Measles	24 months without active GVHD or on immunosuppression	1
Rubella	24 months without active GVHD or on immunosuppression	1
Mumps	24 months without active GVHD or on immunosuppression	1
Varicella	24 months without active GVHD or on immunosuppression	1
BCG	not vaccinate	
Japanese B encephalitis	when travelling to endemic areas	

[a] >2 years of age
[b] Family members of HBs antigen positive carrier
[c] <13 years of age, two doses are recommended

Table 3 Our criteria to initiate live attenuated vaccines since April 2009

1. More than 24 months after transplant
2. No active chronic GVHD and immunosuppressive therapy
3. Less than 6 years old: lymphocyte count >1,500/μl or CD4 cell count >700/μl
   6 years old or more: lymphocyte count >1,000/μl or CD4 cell count >500/μl
4. Phytohemagglutinin response (PHA) normal and serum IgG >500 mg/dl
5. More than 3 months after any transfusions and intravenous immunoglobulin and more than 6 months after high dose intravenous immunoglobulin

除くと，ドナーソースが骨髄血に比べて臍帯血で抗体陽性率が高い傾向にあると考えられた。

7. 国内外でのガイドラインの相違

海外のガイドラインは小児に対しては始めからMMRは2回接種を推奨しており，その有効性も報告されている。移植後18か月と24か月にMMR接種を行った結果，予防接種前の麻疹抗体陽性率が60%であったのが，1回目のワクチン接種，2回目のワクチン接種後の抗体陽性率は91%，100%であった[24]。

また海外のガイドラインにおいて水痘ワクチンは任意で，帯状疱疹ワクチンは推奨しないとしている[1]。推奨されない理由としてはその安全性を示す根拠に乏しいことがあげられている。しかし，限られた症例数のなかでは安全であったことが報告されており，移植後ワクチン接種の時期がそれぞれ中央値32か月と48か月での抗体陽転率は，単回接種で55.6%，63.6%であり，再接種で64.3%，86.4%であった[25,26]。近年では造血幹細胞移植経験者に対し水痘ワクチンよりさらに強い力価をもつ帯状疱疹ワクチン接種の経験が報告された[27]。移植後24か月が経過しており，GVHDがなく，免疫抑制剤の服用もない110名にワクチン接種を行った。2例で水痘様発疹がみられたが，ワクチン株によるものか野生株によるものかは不明で，抗ウイルス薬の投与を受けて治癒したことから，基本的には安全であるとしている。この論文で使用された帯状疱疹ワクチンはZostavax[®]で，日本で一般的に使用されている水痘ワクチンと同様の水痘・帯状疱疹ウイルス「岡株」が使用されており，その力価も同等である[28]。我が国での生活環境におけるその罹患率や移植後の帯状疱疹発症率を鑑みると，臨床上はもっとも接種すべきワクチンのひとつではないかと考えられるが，その有効性に対するエビデンスにはまだ乏しく今後の報告が待たれる。

8. 結　　語

造血幹細胞移植後の長期生存者が増えていくなか，その長期フォローアップの一環としてのVPD予防への関心が高まっている。移植経験者へのワクチン接種の安全

性や有効性，また有効な接種方法などまだ定まっていない部分も多く，保険収載などの社会的な問題も残されている。今後多くの研究がなされ，移植経験者が不要なVPDに罹患し生活の質を損なうことのないように標準的なワクチン接種計画が確立されることが望まれる。

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著者のCOI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

文　献

1) Ljungman P, Cordonnier C, Einsele H, et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant. 2009; **44**: 521-526.
2) Engelhard D, Cordonnier C, Shaw PJ, et al. Early and late invasive pneumococcal infection following stem cell transplantation: a European Bone Marrow Transplantation survey. Br J Haematol. 2002; **117**: 444-450.
3) Nakano T, Shimono Y, Sugiyama K, et al. Clinical features of measles in immunocompromised children. Acta Paediatr Jpn. 1996; **38**: 212-217.
4) Koc Y, Miller KB, Schenkein DP, et al. Varicella zoster virus infections following allogeneic bone marrow transplantation: frequency, risk factors, and clinical outcome. Biol Blood Marrow Transplant. 2000; **6**: 44-49.
5) Nichols WG, Guthrie KA, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis. 2004; **39**: 1300-1306.
6) 国立感染症研究所感染症疫学センター．麻疹ウイルス分離・検出状況 2014年（2014年4月28日現在報告数）．(http://www.nih.go.jp/niid/ja/iasr-measles.html). Accessed 2014 June 3.
7) 国立感染症研究所感染症疫学センター．風疹ウイルス分離・検出状況 2012～2014年（2014年5月2日現在）．(http://www.nih.go.jp/niid/ja/iasr-rubella.html). Accessed 2014 June 3.
8) 国立感染症研究所感染症疫学センター．先天性風しん症候群（CRS）の報告（2014年3月5日現在）．(http://www.nih.go.jp/niid/ja/rubella-m-111/700-idsc/4464-rubella-crs-20140305.html). Accessed 2014 March 30.
9) 東英一．造血細胞移植後の麻疹発症に関する全国調査 全年齢層で急増する麻疹による高い死亡率[抄録]．第25回日本造血細胞移植学会抄録集．2002; 148.
10) Ljungman P, Lewensohn-Fuchs I, Hammarström V, et al. Long-term immunity to measles, mumps, and rubella after allogeneic bone marrow transplantation. Blood. 1994; **84**: 657-663.
11) Pauksen K, Duraj V, Ljungman P, et al. Immunity to and immunization against measles, rubella and mumps in patients after autologous bone marrow transplantation. Bone Marrow Transplant. 1992; **9**: 427-432.
12) Ljungman P, Aschan J, Barkholt L, et al. Measles immunity after allogeneic stem cell transplantation; influence of donor type, graft type, intensity of conditioning, and graft-versus host disease. Bone Marrow Transplant. 2004; **34**: 589-593.
13) 花田良二，菊池陽，康勝好，ほか．造血器疾患患者の麻疹抗体価．日小児血液会誌．2002; **16**: 246.
14) 東英一．造血細胞移植後の免疫能．日小児血液会誌．2005; **19**: 566-577.
15) Roux E, Dumont-Girard F, Starobinski M, et al. Recovery of immune reactivity after T-cell-depleted bone marrow transplantation depends on thymic activity. Blood. 2000; **96**: 2299-2303.
16) 日本造血細胞移植学会ガイドライン委員会．造血細胞移植ガイドライン 予防接種．2008年7月．(http://www.jshct.com/guideline/pdf/2008yobousesshu.pdf). Accessed 2014 March 31.
17) Danerseau AM, Robinson JL. Efficacy and safety of measles, mumps, rubella and varicella live viral vaccines in transplant recipients receiving immunosuppressive drugs. World J Pediatr. 2008; **4**: 254-258.
18) Machado CM, de Souza VA, Sumita LM, da Rocha IF, Dulley FL, Pannuti CS. Early measles vaccination in bone marrow transplant recipients. Bone Marrow Transplant. 2005; **35**: 787-791.
19) 堀浩樹．予防接種ガイドライン．JPLSG長期フォローアップ委員会 長期フォローアップガイドライン作成ワーキンググループ(編集)．小児がん治療後の長期フォローアップガイドライン．東京，医薬ジャーナル社; 2013: 312-316.
20) Forlenza CJ, Small TN. Live (vaccines) from New York. Bone Marrow Transplant. 2013; **48**: 749-754.
21) Ljungman P, Fridell E, Lönnqvist B, et al. Efficacy and safety of vaccination of marrow transplant recipients with a live attenuated measles, mumps, and rubella vaccine. J Infect Dis. 1989; **159**: 610-615.
22) Spoulou V, Giannaki M, Vounatsou M, Bakoula C, Grafakos S. Long-term immunity to measles, mumps and rubella after MMR vaccination among children with bone marrow transplants. Bone Marrow Transplant. 2004; **33**: 1187-1190.
23) Inaba H, Hartford CM, Pei D, et al. Longitudinal analysis of antibody response to immunization in paediatric survivors after allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2012; **156**: 109-117.
24) Patel SR, Ortín M, Cohen BJ, et al. Revaccination with measles, tetanus, poliovirus, *Haemophilus influenzae* type B, meningococcus C, and pneumococcus vaccines in children after hematopoietic stem cell transplantation. Clin Infect Dis. 2007; **44**: 625-634.

25) Kussmaul SC, Horn BN, Dvorak CC, Abramovitz L, Cowan MJ, Weintrub PS. Safety of the live, attenuated varicella vaccine in pediatric recipients of hematopoietic SCTs. Bone Marrow Transplant. 2010; **45**: 1602-1606.
26) Chou JF, Kernan NA, Prockop S, et al. Safety and immunogenicity of the live attenuated varicella vaccine following T replete or T cell-depleted related and unrelated allogeneic hematopoietic cell transplantation (alloHCT). Biol Blood Marrow Transplant. 2011; **17**: 1708-1713.
27) Issa NC, Marty FM, Leblebjian H, et al. Live attenuated varicella-zoster vaccine in hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2014; **20**: 285-287.
28) 神谷齊, 浅野喜造, 尾崎隆男, ほか. 水痘ワクチンの力価と流通時のワクチン力価の安定性. 感染症誌. 2011; **85**: 161-165.

bjh research paper

Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan

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Summary

To evaluate haematopoietic stem cell transplantation (HSCT) in children and adolescents, we reviewed the records of 47 patients who were ≤18 years, had relapsed or refractory anaplastic large cell lymphoma, and received HSCT between 1990 and 2010. At HSCT, complete remission (CR) was less common in allogeneic HSCT recipients (n = 24) than in autologous HSCT recipients (n = 23) (P = 0·01). The autologous and allogeneic HSCT groups differed in terms of 5-year event-free survival (EFS) (38% vs. 50%, P = 0·63), cumulative incidence of progress or relapse (49% vs. 28%, P = 0·25), and treatment-related mortality (12% vs. 25%, P = 0·40). However, these differences were not significant. Patients with non-CR at autologous HSCT had a significantly lower EFS rate (14% vs. 48%, P = 0·03). Conversely, although those with non-CR at allogeneic HSCT had a lower EFS rate, this was not significant (44% vs. 63%, P = 0·26). Reduced-intensity conditioning regimens were used for three of the 16 allogeneic HSCTs received by patients with non-CR. These three patients achieved CR, surviving 32–65 months after HSCT. These results demonstrated that allogeneic HSCT might be a treatment option for patients who do not achieve CR through conventional chemotherapy.

Keywords: anaplastic large cell lymphoma, children, adolescents, haematopoietic stem cell transplantation, reduced-intensity conditioning.

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Anaplastic large cell lymphoma (ALCL) is rare in children, accounting for 10–15% of childhood non-Hodgkin lymphoma cases (Murphy, 1994). The event-free survival (EFS) rate is 65–75% in children and adolescents receiving a first-line strategy based on short-pulse chemotherapy over a period of 3–6 months (Brugières *et al*, 1998, 2009a; Seidemann *et al*, 2001; Le Deley *et al*, 2010). Accordingly, the relapse rate is approximately 30% in most study series. The treatment of relapsed and refractory ALCL remains a matter of debate. Patients with relapsed ALCL have a 30–60% chance of survival under current treatment strategies, which include high-dose chemotherapy with haematopoietic stem cell transplantation (HSCT) and long-term treatment with vinblastine (Brugières *et al*, 2000, 2009b; Williams *et al*, 2002; Mori *et al*, 2006; Woessmann *et al*, 2006; Stockklausner *et al*, 2008; Gross *et al*, 2010). In contrast, patients who experience ALCL progression during first-line chemotherapy have extremely poor outcomes (Woessmann *et al*, 2006) and autologous or allogeneic HSCT is required as the most appropriate therapy.

Some evidence is available regarding the roles of autologous and allogeneic HSCT in paediatric ALCL. However, data are limited to several HSCT case series and case reports. In particular, few reports have been published regarding allogeneic HSCT for paediatric ALCL. We previously reported a retrospective analysis of 26 paediatric patients with recurrent ALCL in Japan (Mori *et al*, 2006). In that study, only three of the eight patients who received autologous HSCT while in their second complete remission (CR) survived without further relapse. In contrast, all six patients who received allogeneic HSCT while in their second CR survived without further relapse. However, our previous study included too few patients for us to discuss the efficacy of HSCT for relapsed or refractory childhood ALCL.

In the present study, we sought to evaluate the efficacy of HSCT for relapsed or refractory ALCL in children and adolescents. We performed a further retrospective analysis of 47 patients who received autologous or allogeneic HSCT for relapsed or refractory ALCL between 1990 and 2010.

Patients and methods

Patients and transplantations

This study was approved by the institutional ethics committee of National Kyushu Cancer Centre. Data on patients who had undergone HSCT were collected from the registries belonging to the Transplant Registry Unified Management Program system of the Japan Society for Hematopoietic Cell Transplantation. The study included 47 patients who had a diagnosis of relapsed or refractory ALCL and received HSCT at age ≤18 years between March 1990 and September 2010. Twenty-three patients received autologous HSCT and 24 patients received allogeneic HSCT. Refractory disease was defined as progression during fist-line treatment. Reduced-intensity conditioning (RIC) regimens were defined as (a) total body irradiation of ≤500 cGy as a single fraction or ≤800 cGy if fractionated, (b) <9 mg/kg of busulfan, (c) ≤180 mg/m^2 of melphalan, (d) <10 mg/kg of thiotepa, or (e) the BEAM regimen (carmustine, etoposide, cytarabine and melphalan), according to previous reports (Yaniv & Stein, 2008; Giralt *et al*, 2009; Ohta *et al*, 2010; Luger *et al*, 2012). All other conditioning regimens were defined as myeloablative conditioning (MAC) regimens.

Statistical analysis

Overall survival (OS), EFS, cumulative incidences of relapse and treatment-related mortality (TRM) were estimated using the Kaplan–Meier method. The Mann–Whitney *U* test, χ^2-test, and Fisher's exact test were used to assess differences in patient characteristics. The level of statistical significance was set at $P < 0{\cdot}05$. All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Autologous HSCT

The patients' characteristics are shown in Table I. Twenty-three patients received autologous HSCT for relapsed or refractory disease as their first transplantation. The median follow-up duration for survivors after autologous HSCT was 154 (range: 9–224) months. The median age at HSCT was 15 (range: 7–18) years. Sixteen patients had achieved CR at HSCT and seven patients had residual disease. Bone marrow and peripheral blood were the stem cell sources in three and 20 patients, respectively. Engraftment was observed in 23 (100%) cases, occurring at a median of 12 d. The 5-year cumulative incidence of relapse was 49% ± 11% (Fig 1A). Treatment-related death occurred in three of the patients who received autologous HSCT and the 5-year cumulative incidence of TRM was 12% ± 9% (Fig 1B). Two of the three patients died of infectious complications and one patient died of multiple organ failure. The 5-year OS and EFS rates were 51% ± 11% and 38% ± 10%, respectively (Fig 2A, B). We observed 5-year EFS rates of 48% ± 13% and 14% ± 13% for patients with CR and non-CR, respectively, at autologous HSCT (Fig 3A), which constituted a significant difference ($P = 0{\cdot}03$).

Allogeneic HSCT

Twenty-four patients received allogeneic HSCT for relapsed or refractory disease (Table I). The median follow-up duration for survivors after allogeneic HSCT was 68 (range: 32–212) months. The median age at HSCT was 13·5 (range: 3–18) years. Of the 24 patients, four had received previous autologous HSCT. Eight patients had achieved CR at HSCT and 16 patients had residual disease (Table I). The sources of stem cells were bone marrow in 13 patients, cord blood in

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Table I. Characteristics of patients with relapsed or refractory ALCL according to the receipt of autologous or allogeneic HSCT.

	Autologous	Allogeneic	*P*
Patients (*n*)	23	24	
Age at HSCT (years)			
Median	15	13·5	0·27
Range	7–18	3–18	
Sex			
Male	17	21	0·24
Female	6	3	
Stage at diagnosis			
I	1	0	0·36
II	3	4	
III	11	6	
IV	4	8	
Unknown	4	6	
Disease status at HSCT			
CR2/CR≥3	14/2	5/3	0·01
Non-CR	7	16	
Conditioning			
TBI/TLI based	7/1	17/1	0·06
Non-TBI based	15	6	
Stem cell source			
BM	3	13	
PB	20	5	
CB	0	6	
Donor			
MRD	–	7	
MUD	–	2	
MMRD	–	6	
MMUD	–	7	
Unknown	–	2	

HSCT, haematopoietic stem cell transplantation; CR, complete remission; BM, bone marrow; CB, cord blood; PB, peripheral blood; MRD, matched related donor; MUD, matched unrelated donor; MMRD, mismatched related donor; MMUD, mismatched unrelated donor; TBI, total body irradiation; TLI, total lymphoid irradiation.

six patients and peripheral blood in five patients. Seven patients had human leucocyte antigen (HLA)-matched related donors, and two patients received stem cells from HLA-matched unrelated donors. Thirteen patients had HLA-mismatched donors. Engraftment was observed in 21 (88%) cases, occurring at a median of 17 d. Two patients died of infection and one died of disease progression before engraftment. The 5-year cumulative incidence of relapse was 28% ± 10% (Fig 1A). Treatment-related death occurred in five patients; four patients died of infectious complications and one patient died of acute graft-*versus*-host disease (GVHD). The 5-year cumulative incidence of TRM was 25% ± 10% (Fig 1B). Acute GVHD of any grade occurred in 13 patients, nine of whom had grade II–IV GVHD. The 5-year OS and EFS rates were 54% ± 10% and 50% ± 10%, respectively (Fig 2A, B). Seven of 24 patients had multiple relapses before their HSCT; the 5-year EFS rates among patients with and without multiple relapses were 43% ± 19% and 53% ± 12%, respectively ($P = 0·67$). We observed 5-year EFS rates of 63% ± 17% and 44% ± 12% among patients with CR and those with non-CR respectively, at allogeneic HSCT (Fig 3B), which did not constitute a significant difference ($P = 0·13$).

At HSCT, CR was less common among allogeneic HSCT recipients than it was among autologous HSCT recipients ($P = 0·01$). However, there were no significant differences between the autologous and allogeneic HSCT patients in terms of cumulative incidence of relapse ($P = 0·25$), cumulative incidence of TRM ($P = 0·40$), 5-year OS ($P = 0·95$) or 5-year EFS ($P = 0·63$).

RIC regimens

Of the 24 patients in the allogeneic group, four underwent allogeneic HSCT using RIC. Their outcomes are shown in Table II. One of the four patients died of bacterial infection and the other three patients survived in CR without relapse after allogeneic HSCT. Interestingly, none of these three patients were in CR at HSCT.

Discussion

Currently, the efficacy and toxicity of HSCT are poorly defined for childhood cases of relapsed or refractory ALCL. Evidence is especially lacking in regards to the efficacy and toxicity of allogeneic HSCT. The present study included 23 patients who underwent autologous HSCT and 24 patients who underwent allogeneic HSCT. Each of the patients was a child or adolescent who had relapsed or refractory ALCL and underwent HSCT in Japan. This report comprises the largest cohort concerning allogeneic HSCT for relapsed or refractory ALCL in childhood.

The Berlin-Frankfürt-Münster (BFM) cohort had efficacies of autologous HSCT (77% OS and 59% EFS among 39 children with relapsed ALCL) that lie at or above the upper range of previously reported series (Woessmann *et al*, 2011). In national case series from the United Kingdom and France, one of six and nine of 15 patients stayed in continuous CR (Brugières *et al*, 2000; Williams *et al*, 2002; Woessmann *et al*, 2011). The Center for International Blood and Marrow Transplant Research (CIBMTR) has reported another large series of autologous HSCTs that were performed for ALCL, noting an EFS of 35% in 24 patients (Gross *et al*, 2010). Previously, we have reported a retrospective analysis of relapsed ALCL, which included 26 patients in Japan (Mori *et al*, 2006). Three of the eight patients who underwent autologous HSCT survived in continuous CR. In the current study, the 5-year OS rate, EFS rate and cumulative incidence of relapse among the 23 patients who underwent autologous HSCT were 51%, 38% and 49%, respectively. These results are similar to the findings of a previous CIBMTR report (Gross *et al*, 2010). In a study of 64 adult and paediatric cases of autologous HSCT for ALCL, Fanin *et al* (1999) reported that disease status at HSCT

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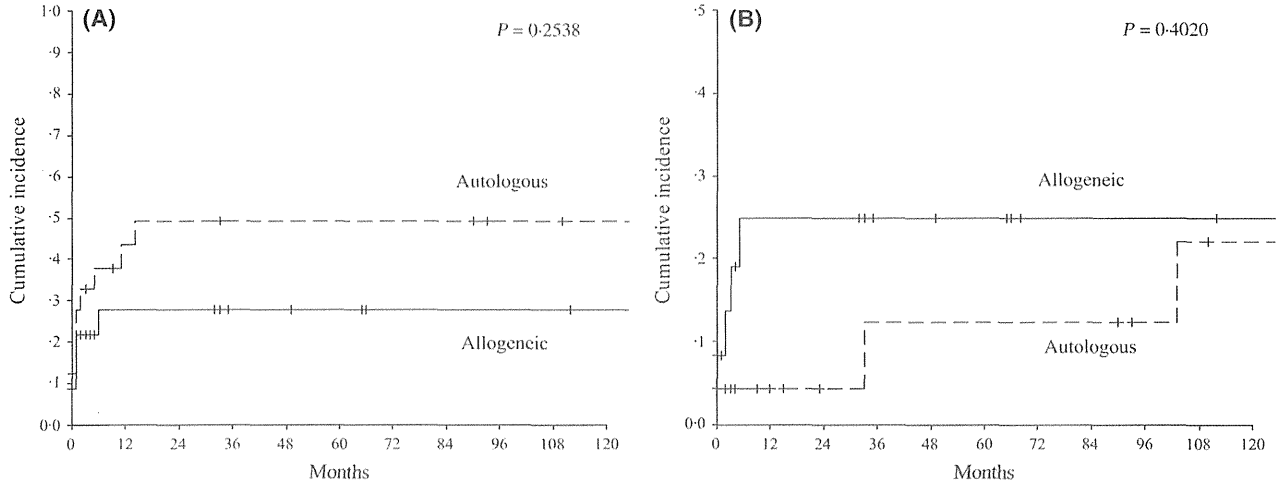

Fig 1. The cumulative incidence of relapse (A) and treatment-related mortality (B) according to autologous and allogeneic haematopoietic stem cell transplantation.

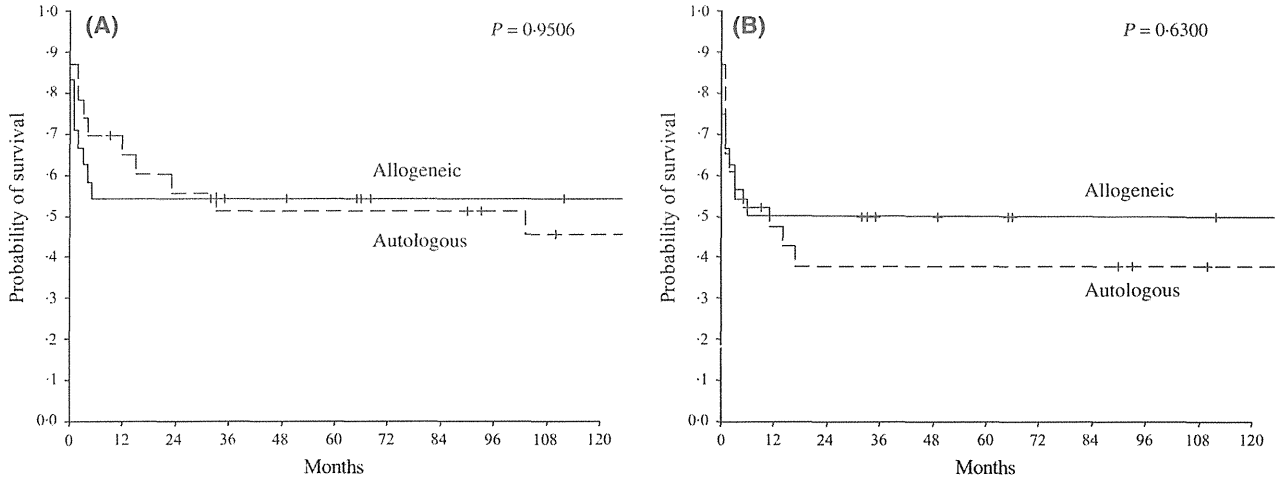

Fig 2. Overall survival (A) and event-free survival (B) according to autologous and allogeneic haematopoietic stem cell transplantation.

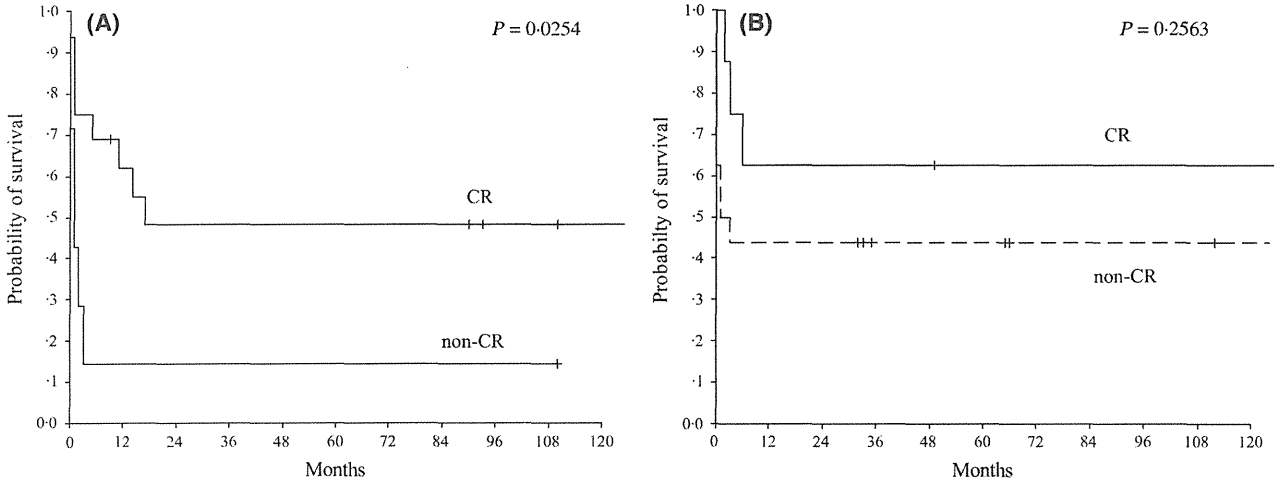

Fig 3. Event-free survival according to disease status at HSCT. (A) Autologous HSCT, (B) allogeneic HSCT. HSCT, haematopoietic stem cell transplantation; CR complete remission.

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Table II. Details and outcomes of patients treated with reduced intensity conditioning and allogeneic HSCT.

Patients	Status at HSCT	Age at HSCT (years)	Donor	Stem cell source	Conditioning regimen	GVHD prophylaxis	aGVHD (Grade)	Extensive cGVHD	Outcome	Follow-up (months)
1	PR	3	UD	CB	TLI 2 Gy, Flu, Mel	Tac, MTX	III	–	CR	32
2	PR	9	UD	CB	Flu, Mel	Tac, MTX	II	–	CR	65
3	CR	18	UD	BM	Flu, Mel, ATG	Tac, MTX	0	NA	TRM	5
4	PR	16	UD	BM	Bu, Flu	Tac, MTX	III	+	CR	33

HSCT, haematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; UD, unrelated donor; BM, bone marrow; CB, cord blood; TLI, total lymphoid irradiation; Bu, busulfan; Flu, fludarabine; Mel, melphalan; ATG, antithymocyte globulin; GVHD, graft-*versus*-host disease; Tac, tacrolimus; MTX, methotrexate; aGVHD, acute GVHD; cGVHD, chronic GVHD; TRM, treatment-related mortality; NA, not applicable.

had predictive value for OS and EFS. In the current study, the EFS of the patients with CR at autologous HSCT was significantly higher than that of the patients with non-CR at autologous HSCT. Brugières *et al* (2000) reported that an interval of <12 months between diagnosis and relapse was associated with a higher risk of failure for the treatment of relapsed ALCL, including autologous HSCT. However, our cohort did not provide sufficient data to compare the risk of failure with the interval between diagnosis and relapse.

The role of allogeneic HSCT has not been defined for cases of childhood ALCL. The currently available evidence is limited to a few reports. The BFM group reported a series of 20 paediatric patients who underwent allogeneic HSCT for relapsed or refractory ALCL, finding a 75% 3-year EFS (Woessmann *et al*, 2006). Twelve of the patients in this study were in CR at HSCT. The CIBMTR has reported another large series of allogeneic HSCTs that were performed for ALCL, observing an EFS of 46% for 12 relapsed or refractory patients (Gross *et al*, 2010). Giulino-Roth *et al* (2013) also reported the cases of 13 paediatric patients with ALCL, eight of whom underwent autologous HSCT and five of whom underwent allogeneic HSCT. The OS and disease-free survival rates were 83% and 77%, respectively. Although our previous study noted that all six patients who underwent allogeneic HSCT during their second CR survived without further relapse (Mori *et al*, 2006), 5-year OS and EFS rates were limited to 54% and 50% in the present study. Patients who underwent allogeneic HSCT while in CR accounted for only eight of the 24 cases. Indeed, the rate of CR at HSCT was lower in the current study than in previous reports of allogeneic HSCT. In the present study, we found no significant difference in EFS according to disease status (CR or non-CR) at allogeneic HSCT. However, the low CR rate at allogeneic HSCT might be associated with the survival rate in the current study, which was lower than the rates noted in previous reports.

In the present study, we observed a 25% TRM rate among patients who underwent allogeneic HSCT for relapsed and refractory disease. Although the cumulative incidence of TRM for allogeneic HSCT was higher than that for autologous HSCT, the difference was not significant ($P = 0{\cdot}40$) (Fig 1B). Several investigations have shown that RIC followed by allogeneic HSCT has the potential to reduce TRM and long-term toxicity in cases of malignant and non-malignant diseases (Carella *et al*, 2000; Dreger *et al*, 2003; Jacobsohn *et al*, 2004; Bradley *et al*, 2007). The BFM cohort of allogeneic HSCTs included one case in which an RIC regimen was administered to a patient with ALCL. The RIC regimen comprised total lymphoid irradiation (2 Gy), fludarabine and melphalan (Brugières *et al*, 2000). Another case in which an RIC regimen [thraco-abdominal irradiation (2 Gy), fludarabine and melphalan] was used has also been reported (Ohta *et al*, 2010). Both of these patients survived in continuous CR following allogeneic HSCT. In the present study, four patients received an RIC regimen followed by allogeneic HSCT. Of these four patients, three were in non-CR at allogeneic HSCT, yet survived in CR for 32–65 months without relapse after HSCT. These results suggest that RIC for relapsed or refractory ALCL may be useful in cases involving allogeneic HSCT, regardless of disease status. However, there are only a few reports of allogeneic HSCT using an RIC regimen for paediatric ALCL. Further evaluations of the efficacy of RIC are necessary and should include larger numbers of patients and a prospective design.

The treatment of relapsed or refractory ALCL remains a matter of debate. Recent studies have reported the efficacies of second-line treatments for relapsed or refractory ALCL, including vinblastine monotherapy, brentuximab vedotin and crizotinib. Brugières *et al* (2009b) studied 36 paediatric patients treated with weekly vinblastine for relapsed or refractory ALCL, finding that this treatment was highly efficacious, with a CR rate of 83%. Furthermore, the 5-year EFS rate was 30%, at which time all but two of the patients had stopped vinblastine for more than 2 years. In adults, a phase II trial of brentuximab vedotin was conducted in patients with relapsed or refractory systemic ALCL. Fifty of 58 patients (86%) achieved an objective response, including 33 patients (57%) in CR (Pro *et al*, 2012). The Children's Oncology Group reported a phase I study of crizotinib for paediatric patients with refractory ALCL, finding that seven of nine children acheived CR following crizotinib monotherapy (Mossé *et al*, 2013). Autologous and allogeneic HSCTs are associated with high rates of toxicities and TRM. Consequently, it will be necessary to speculate about the selection of second-line treatments for relapsed or refractory ALCL in children and adolescents.

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In conclusion, both autologous and allogeneic HSCT can offer the prospect of durable disease-free survival for relapsed and refractory ALCL in childhood and adolescence. Patients with CR at the time of autologous HSCT had significantly greater EFS than patients with non-CR at the time of autologous HSCT. Our results suggest that allogeneic HSCT might provide a better outcome for patients who are resistant to chemotherapy after relapse, and those with non-CR at the time of HSCT. Furthermore, an RIC regimen followed by allogeneic HSCT might even be useful for these patients. However, the small number of patients in our cohort prevented us from investigating the efficacy of allogeneic HSCT with an RIC regimen. In the new era of molecular target drugs, the best candidates for autologous and allogeneic HSCT remain to be clarified by further analyses and prospective studies of relapsed or refractory ALCL in childhood and adolescence.

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Author contributions

R Kobayashi, T Mori and R Fukano designed the research study; M Chin, H Goto, Y Takahashi, J Hara, YD Park, M Inoue, Y Koga, J Inagaki, H Sakamaki, S Adachi, K Kawa, K Kato and R Suzuki collected the data; R Fukano analysed the data and wrote the paper. All authors reviewed the manuscript.

Conflict of interest

There are no conflicts of interest to declare.

References

Bradley, M.B., Satwani, P., Baldinger, L., Morris, E., van de Ven, C., Del Toro, G., Garvin, J., George, D., Bhatia, M., Roman, E., Baxter-Lowe, L.A., Schwartz, J., Qualter, E., Hawks, R., Wolownik, K., Foley, S., Militano, O., Leclere, J., Cheung, Y.K. & Cairo, M.S. (2007) Reduced intensity allogeneic umbilical cord blood transplantation in children and adolescent recipients with malignant and non-malignant diseases. *Bone Marrow Transplantation*, **40**, 621–631.

Brugières, L., Deley, M.C., Pacquement, H., Meguerian-Bedoyan, Z., Terrier-Lacombe, M.J., Robert, A., Pondarré, C., Leverger, G., Devalck, C., Rodary, C., Delsol, G. & Hartmann, O. (1998) CD30(+) anaplastic large-cell lymphoma in children: analysis of 82 patients enrolled in two consecutive studies of the French Society of Pediatric Oncology. *Blood*, **92**, 3591–3598.

Brugières, L., Quartier, P., Le Deley, M.C., Pacquement, H., Perel, Y., Bergeron, C., Schmitt, C., Landmann, J., Patte, C., Terrier-Lacombe, M.J., Delsol, G. & Hartmann, O. (2000) Relapses of childhood anaplastic large-cell lymphoma: treatment results in a series of 41 children—A report from the French Society of Pediatric Oncology. *Annals of Oncology*, **11**, 53–58.

Brugières, L., Le Deley, M.C., Rosolen, A., Williams, D., Horibe, K., Wrobel, G., Mann, G., Zsiros, J., Uyttebroeck, A., Marky, I., Lamant, L. & Reiter, A. (2009a) Impact of the methotrexate administration dose on the need for intrathecal treatment in children and adolescents with anaplastic large-cell lymphoma: results of a randomized trial of the EICNHL Group. *Journal of Clinical Oncology*, **27**, 897–903.

Brugières, L., Pacquement, H., Le Deley, M.C., Leverger, G., Lutz, P., Paillard, C., Baruchel, A., Frappaz, D., Nelken, B., Lamant, L. & Patte, C. (2009b) Single-drug vinblastine as salvage treatment for refractory or relapsed anaplastic large-cell lymphoma: a report from the French Society of Pediatric Oncology. *Journal of Clinical Oncology*, **27**, 5056–5061.

Carella, A.M., Cavaliere, M., Lerma, E., Ferrara, R., Tedeschi, L., Romanelli, A., Vinci, M., Pinotti, G., Lambelet, P., Loni, C., Verdiani, S., De Stefano, F., Valbonesi, M. & Corsetti, M.T. (2000) Autografting followed by nonmyeloablative immunosuppressive chemotherapy and allogeneic peripheral-blood hematopoietic stem-cell transplantation as treatment of resistant Hodgkin's disease and non-Hodgkin's lymphoma. *Journal of Clinical Oncology*, **18**, 3918–3924.

Dreger, P., Brand, R., Hansz, J., Milligan, D., Corradini, P., Finke, J., Deliliers, G.L., Martino, R., Russell, N., Van Biezen, A., Michallet, M. & Niederwieser, D. (2003) Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. *Leukemia*, **17**, 841–848.

Fanin, R., Ruiz de Elvira, M.C., Sperotto, A., Baccarani, M. & Goldstone, A. (1999) Autologous stem cell transplantation for T and null cell CD30-positive anaplastic large cell lymphoma: analysis of 64 adult and paediatric cases reported to the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplantation*, **23**, 437–442.

Giralt, S., Ballen, K., Rizzo, D., Bacigalupo, A., Horowitz, M., Pasquini, M. & Sandmaier, B.; Reduced-intensity conditioning regimen workshop: defining the dose spectrum. (2009) Report of a workshop convened by the center for international blood and marrow transplant research. *Biology of Blood and Marrow Transplantation*, **15**, 367–369.

Giulino-Roth, L., Ricafort, R., Kernan, N.A., Small, T.N., Trippett, T.M., Steinherz, P.G., Prockop, S.E., Scaradavou, A., Chiu, M., O'Reilly, R.J. & Boulad, F. (2013) Ten-year follow-up of pediatric patients with non-Hodgkin lymphoma treated with allogeneic or autologous stem cell transplantation. *Pediatric Blood & Cancer*, **60**, 2018–2024.

Gross, T.G., Hale, G.A., He, W., Camitta, B.M., Sanders, J.E., Cairo, M.S., Hayashi, R.J., Termuhlen, A.M., Zhang, M.J., Davies, S.M. & Eapen, M. (2010) Hematopoietic stem cell transplantation for refractory or recurrent non-Hodgkin lymphoma in children and adolescents. *Biology of Blood and Marrow Transplantation*, **16**, 223–230.

Jacobsohn, D.A., Duerst, R., Tse, W. & Kletzel, M. (2004) Reduced intensity haemopoietic stem-cell transplantation for treatment of non-malignant diseases in children. *Lancet*, **364**, 156–162.

Le Deley, M.C., Rosolen, A., Williams, D.M., Horibe, K., Wrobel, G., Attarbaschi, A., Zsiros, J., Uyttebroeck, A., Marky, I.M., Lamant, L., Woessmann, W., Pillon, M., Hobson, R., Mauguen, A., Reiter, A. & Brugières, L. (2010) Vinblastine in children and adolescents with highrisk anaplastic large-cell lymphoma: results of the randomized ALCL99-vinblastine trial. *Journal of Clinical Oncology*, **28**, 3987–3993.

Luger, S.M., Ringdén, O., Zhang, M.J., Pérez, W.S., Bishop, M.R., Bornhauser, M., Bredeson, C.N., Cairo, M.S., Copelan, E.A., Gale, R.P., Giralt, S.A., Gulbas, Z., Gupta, V., Hale, G.A., Lazarus, H.M., Lewis, V.A., Lill, M.C., McCarthy, P.L., Weisdorf, D.J. & Pulsipher, M.A. (2012) Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. *Bone Marrow Transplantation*, **47**, 203–211.

Mori, T., Takimoto, T., Katano, N., Kikuchi, A., Tabuchi, K., Kobayashi, R., Ayukawa, H., Kumagai, M.A., Horibe, K. & Tsurusawa, M. (2006) Recurrent childhood anaplastic large cell lymphoma: a retrospective analysis of registered cases in Japan. *British Journal of Haematology*, **132**, 594–597.

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Mossé, Y.P., Lim, M.S., Voss, S.D., Wilner, K., Ruffner, K., Laliberte, J., Rolland, D., Balis, F.M., Maris, J.M., Weigel, B.J., Ingle, A.M., Ahern, C., Adamson, P.C. & Blaney, S.M. (2013) Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncology*, **14**, 472–480.

Murphy, S.B. (1994) Paediatric lymphomas: recent advances and commentary on Ki-1-positive anaplastic large-cell lymphomas of childhood. *Annals of Oncology*, **5**, 31–33.

Ohta, H., Kusuki, S., Yoshida, H., Sato, E., Hashii, Y. & Ozono, K. (2010) Allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning for a child with recurrent anaplastic large cell lymphoma. *International Journal of Hematology*, **92**, 190–193.

Pro, B., Advani, R., Brice, P., Bartlett, N.L., Rosenblatt, J.D., Illidge, T., Matous, J., Ramchandren, R., Fanale, M., Connors, J.M., Yang, Y., Sievers, E.L., Kennedy, D.A. & Shustov, A. (2012) Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. *Journal of Clinical Oncology*, **30**, 2190–2196.

Seidemann, K., Tiemann, M., Schrappe, M., Yakisan, E., Simonitsch, I., Janka-Schaub, G., Dörffel, W., Zimmermann, M., Mann, G., Gadner, H., Parwaresch, R., Riehm, H. & Reiter, A. (2001) Short-pulse B-non-Hodgkin lymphoma-type chemotherapy is efficacious treatment for pediatric anaplastic large cell lymphoma: a report of the Berlin-Frankfurt- Munster Group Trial NHL-BFM 90. *Blood*, **97**, 3699–3706.

Stockklausner, C., Behnisch, W., Mechtersheimer, G., Möller, P. & Kulozik, A.E. (2008) Long-term remission of children with relapsed and secondary anaplastic large cell non-Hodgkin lymphoma (ALCL) following treatment with pulsed dexamethasone and low dose etoposide. *Paediatric Blood & Cancer*, **50**, 126–129.

Williams, D.M., Hobson, R., Imeson, J., Gerrard, M., McCarthy, K. & Pinkerton, C.R. (2002) Anaplastic large cell lymphoma in childhood: analysis of 72 patients treated on The United Kingdom Children's Cancer Study Group chemotherapy regimens. *British Journal of Haematology*, **117**, 812–820.

Woessmann, W., Peters, C., Lenhard, M., Burkhardt, B., Sykora, K.W., Dilloo, D., Kremens, B., Lang, P., Führer, M., Kühne, T., Parwaresch, R., Ebell, W. & Reiter, A. (2006) Allogeneic haematopoietic stem cell transplantation in relapsed or refractory anaplastic large cell lymphoma of children and adolescents: a Berlin- Frankfurt-Munster group report. *British Journal of Haematology*, **133**, 176–182.

Woessmann, W., Zimmermann, M., Lenhard, M., Burkhardt, B., Rossig, C., Kremens, B., Lang, P., Attarbaschi, A., Mann, G., Oschlies, I., Klapper, W. & Reiter, A. (2011) Relapsed or refractory anaplastic large-cell lymphoma in children and adolescents after Berlin-Frankfurt-Muenster (BFM)-type first-line therapy: a BFM-group study. *Journal of Clinical Oncology*, **29**, 3065–3071.

Yaniv, I. & Stein, J. (2008) Reduced-intensity conditioning in children: a reappraisal in 2008. *Bone Marrow Transplantation*, **41** (Suppl 2), S18–S22.

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Peripheral blood lymphocyte telomere length as a predictor of response to immunosuppressive therapy in childhood aplastic anemia

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ABSTRACT

Predicting the response to immunosuppressive therapy could provide useful information to help the clinician define treatment strategies for patients with aplastic anemia. In our current study, we evaluated the relationship between telomere length of lymphocytes at diagnosis and the response to immunosuppressive therapy in 64 children with aplastic anemia, using flow fluorescence *in situ* hybridization. Median age of patients was ten years (range 1.5-16.2 years). Severity of the disease was classified as very severe in 23, severe in 21, and moderate in 20 patients. All patients were enrolled in multicenter studies using antithymocyte globulin and cyclosporine. The response rate to immunosuppressive therapy at six months was 52% (33 of 64). The probability of 5-year failure-free survival and overall survival were 56% (95% confidence interval (CI): 41-69%) and 97% (95%CI: 87-99%), respectively. Median telomere length in responders was -0.4 standard deviation (SD) (-2.7 to +3.0 SD) and -1.5 SD (-4.0 to +1.6 (SD)) in non-responders (P<0.001). Multivariate analysis showed that telomere length shorter than -1.0 SD (hazard ratio (HR): 22.0; 95%CI: 4.19-115; P<0.001), platelet count at diagnosis less than 25x10^9/L (HR: 13.9; 95%CI: 2.00-96.1; P=0.008), and interval from diagnosis to immunosuppressive therapy longer than 25 days (HR: 4.81; 95%CI: 1.15-20.1; P=0.031) were the significant variables for poor response to immunosuppressive therapy. Conversely to what has been found in adult patients, measurement of the telomere length of lymphocytes at diagnosis is a promising assay in predicting the response to immunosuppressive therapy in children with aplastic anemia.

Introduction

Aplastic anemia (AA) is defined as bone marrow aplasia and peripheral blood pancytopenia; disease pathogenesis is thought to involve immune-mediated processes. The first choice of treatment for severe AA in children is hematopoietic stem cell transplantation from a human leukocyte antigen (HLA)-matched sibling donor.[1,2] However, 60-70% of children with severe AA have no matched sibling donor and receive immunosuppressive therapy (IST), consisting of antithymocyte globulin (ATG) and cyclosporine (CyA). According to previous studies in children, the response rate to IST at six months was 60-70%, with the probability of survival at five years being over 90%. On the other hand, relapses occur in 10-30% of patients who responded to IST and, overall, clonal evolution develops in 10-15% patients.[3-5] In adults, several pre-treatment biomarkers have been proposed as promising tests for predicting favorable response to IST, including the presence of either human leukocyte antigen (HLA)-DR15 or a minor population of paroxysmal nocturnal hemoglobinuria (PNH)-type cells.[6-9] However, we previously reported that neither test was useful to predict response to IST and that lower white blood cell count and shorter interval from diagnosis to IST were significant predictive markers of better response,[10] on the other hand, a National Institutes of Health (NIH) study showed that higher base-line absolute reticulocyte and lymphocyte counts were highly predictive of response to IST in adult patients.[11] These results suggest a difference in etiology of AA between adults and children.[10]

Dyskeratosis congenita (DC) is a rare inherited disease characterized by the classical mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and mucosal leukoplakia.[12] Patients with DC are unable to maintain the telomere complex that are protein-DNA structures at the end of eukaryotic chromosomes that prevent degradation and aberrant recombination of the chromosome ends,[13,14] and consequently have very short telomeres.[15] Shortened telomeres can cause a wide variety of clinical features consisting not only of

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mucocutaneous abnormalities, but also other symptoms, including bone marrow failure, pulmonary fibrosis, hepatic fibrosis, and predisposition to malignancy.[16] Several recent studies revealed cryptic forms of DC among patients with seemingly acquired AA who did not have apparent physical abnormalities.[17,18] Failure of AA patients to respond to IST may be explained by the presence of cryptic inherited bone marrow failure syndromes (IBMFSs).

Several investigators have demonstrated that telomere lengths of leukocytes in patients with AA vary widely, with an increased proportion of the patients having shorter telomeres than healthy individuals.[19,20] It is known that not only patients with typical DC, but also those with cryptic DC have very short telomeres.[21] Moreover, the telomere length in leukocytes is decreased in subsets of patients with other IBMFSs including Fanconi anemia,[22] Diamond-Blackfan anemia,[23] and Schwachman-Diamond syndrome.[24] Therefore, measuring telomere length of patients with AA at diagnosis may be useful in detecting patients with cryptic type of IBMFSs.

Recently, Scheinberg *et al.* reported that the telomere length of peripheral blood leukocytes was associated with risk of hematologic relapse, clonal evolution to myelodysplastic syndrome, and overall survival (OS), but not related to hematologic response to IST in patients with severe AA.[25] Because there was no study to validate their observation, we evaluated the relationship between telomere length in hematopoietic cells before IST and the response to IST in children with AA.

Methods

Patients

Peripheral blood samples at diagnosis and clinical records were obtained from 64 children who fulfilled entry criteria and enrolled in two prospective studies conducted by the Japan Childhood Aplastic Anemia Study Group.[26,27] Patients with acquired AA were eligible if selection criteria were satisfied (see *Online Supplementary Appendix* for details). Thirty-eight patients received horse ATG (Lymphoglobulin; Genzyme, Cambridge, MA, USA) at 15 mg/kg/day for five days and 26 received rabbit ATG (Thymoglobulin, Genzyme, Cambridge, MA, USA) at 3.75 mg/kg/day for five days. CyA (6 mg/kg/day, orally) was started on Day 1 and continued to at least Day 180. The dose was adjusted to achieve a whole blood trough level of 100-200 ng/mL. Standard supportive care was supplied in each institute. Response to IST was evaluated according to previously described criteria.[3] We defined patients with complete response or partial response at six months after IST as responders, and the other patients as non-responders. Relapse was defined by conversion to no response from a partial or complete response and/or the requirement for blood transfusions.

All samples and clinical records were collected after written informed consent had been obtained according to protocols approved by the Ethics Review Committee, Nagoya University Graduate School of Medicine (Research n. 732).

Measurements of telomere length and population of PNH clones

The average relative telomere length (RTL) of peripheral lymphocytes was measured by flow fluorescence *in situ* hybridization (flow-FISH), using a Telomere PNA kit (Dako Cytomation, Glostrup, Denmark).[28] Lymphocytes were derived from fresh peripheral blood in 38 cases and from frozen stored peripheral blood in 26 cases. We used delta RTL to compare patients' telomere length with that of age-matched healthy controls. Details of methods for measuring telomere length and definition of delta RTL are described in the *Online Supplementary Methods*. A minor population of paroxysmal nocturnal hemoglobinuria (PNH)-type granulocytes and red blood cells were also evaluated by flow cytometry according to a previously described method.[10]

Statistical analysis

We analyzed predictive variables associated with response to IST, failure-free survival (FFS; in which relapse, clonal evolution, second IST, HSCT, and death were censored), transplantation-free survival (TFS; in which HSCT and death were censored), and OS. Pre-treatment variables included patient's sex, age, etiology, disease severity, interval from diagnosis to IST, leukocyte count, lymphocyte count, neutrophil count, hemoglobin (Hb) level, platelet count, reticulocyte count, presence of HLA-DR15, presence of minor PNH clone, and delta RTL. Differences in these variables between responders and non-responders were assessed using the Mann-Whitney U-test and Fisher's exact probability test. Predictive factors with $P<0.10$ in the univariate analyses were set in the multivariate analysis (logistic regression modeling). $P<0.05$ was considered statistically significant. Measures of association were expressed as hazard ratios (HR) with 95% confidence intervals (CI). All tests were two-tailed with a type I error of less than 0.05 considered as statistically significant. All analyses were performed using STATA12.0 software (STATA, College Station, TX, USA).

Results

Pre-treatment patients' characteristics and clinical outcomes

A total of 64 patients with AA were included in this study. Patients' characteristics are shown in Table 1. The median age at IST was 10.0 years (range 1.5-16.2 years). Disease severity was assessed as very severe in 23 patients, severe in 21 patients, and moderate in 20 patients. Causes of AA were idiopathic in 60 patients and hepatitis in 4 patients. Median follow-up time from the time of IST was 35 months (range 6-132 months).

Overall, 33 of 64 patients (52%) responded to IST at six months after administration of ATG. Of the 33 responders, 4 children relapsed at 6, 34, 66, and 91 months after IST, respectively. The probability of 5-year cumulative incidence of relapse was 8% (95%CI: 2-28%). Nineteen transplantations were carried out for non-responders or patients with relapse. Of 64 children with AA, only one patient developed clonal evolution at 23 months after IST. During the observation period, 2 patients died; both of them had shown no response to IST, one suffered from lethal cerebral hemorrhage at six months, and the other underwent bone marrow transplantation from an HLA-matched unrelated donor at 12 months after IST and died of transplantation-related hepatic failure. The probability of 5-year FFS, TFS, and OS were 56% (95%CI: 41-69%), 63% (95%CI: 48-75%), and 97% (95%CI: 87-99%), respectively.

Telomere length of children with AA

Comparing SD calculated in 71 healthy individuals, median telomere length was -0.9SD (range -4.0 to +3.0SD) in all patients (n=64), -0.4SD (range -2.7 to +3.0SD) in

Table 1. Patients' characteristics.

Variables		Total	Responder	Non-responder	P
N		64	33	31	
Sex	M/F	38 / 26	22 / 11	16 / 15	NS
Age at diagnosis (range)	median	10.0 (1.5-16.2)	10.0 (1.5-16.2)	9.7 (2.6-15.1)	NS
Severity	VSAA/ SAA/MAA	23/21/20	12/10/11	11/11/9	NS
Etiology	Idiopathic/hepatitis	60/4	31/2	29/2	NS
ATG	Horse/rabbit	38/26	23/10	15/16	0.08
Interval from diagnosis to IST	median (range)	22 (1-341)	18 (1-85)	28 (4-341)	0.02
WBC at diagnosis (x10⁹/L)	median (range)	2300 (20-8700)	2300 (20-8700)	2400 (300-5000)	NS
NEU at diagnosis (x10⁹/L)	median (range)	300 (0-3130)	380 (0-3130)	260 (0-1140)	NS
LYM at diagnosis (x10⁹/L)	median (range)	1900 (20-5600)	1800 (20-5600)	2000 (200-4300)	NS
Hb at diagnosis (g/dL)	median (range)	7.3 (2.7-11.4)	7.2 (2.8-11.0)	7.4 (2.7-11.4)	NS
PLT at diagnosis (x10⁹/L)	median (range)	1.6 (0.3-5.4)	2.1 (0.4-5.2)	1.6 (0.3-5.4)	0.04
RET at diagnosis (x10⁹/L)	median (range)	27 (0-96)	27 (3-96)	27 (0-75)	NS
PNH clone	Positive/negative	11 / 53	7 / 26	4 / 27	NS
HLA-DR15	Positive/negative	20 / 44	13 / 20	7 / 24	NS
delta RTL (SD)	median (range)	-0.9 (-4.0 - +3.0)	-0.4 (-2.8 - +3.0)	-1.5 (-4.0 - +1.6)	<0.001

ATG: antithymocyte globulin; F: female; Hb: hemoglobin; HLA: human leukocyte antigen; IST: immune suppressive therapy; LYM: lymphocyte count; M: male; MAA: moderate aplastic anemia; NEU: neutrophil count; NS: not significant; PLT: platelet count; PNH: paroxysmal nocturnal hemoglobinuria; RET: reticulocyte count; RTL: relative telomere length; SAA: severe aplastic anemia; SD: standard deviation; VSAA: very severe aplastic anemia; WBC: white blood cell count.

Table 2. Multivariate analyses for poor response to IST, failure-free survival, and transplantation-free survival.

	HR	95% CI	P
Multivariate analysis for response to IST			
Interval from diagnosis to IST >25 days	4.81	1.15-20.1	0.031
IST with rabbit ATG	0.79	0.16-3.96	0.77
PLT <25x10⁹/L	13.9	2.00-96.1	0.008
RTL <-1SD	22	4.19-115	<0.001
Multivariate analysis for FFS			
IST with rabbit ATG	1.27	0.47-3.48	0.64
LYM >2.0x10⁹/L	2.32	1.02-5.24	0.044
PLT <25x10⁹/L	4.11	1.17-14.5	0.028
RTL <-1SD	2.01	0.83-4.89	0.12
Multivariate analysis for TFS			
IST with rabbit ATG	1.32	0.45-3.86	0.61
LYM >2.0x10⁹/L	3.42	1.32-8.81	0.011
PLT <25x10⁹/L	4.64	1.00-21.6	0.051
RTL <-1SD	2.84	1.01-7.97	0.048

ATG: antithymocyte globulin; CI: confidence interval; FFS: failure-free survival; HR: hazard ratio; IST: immunosuppressive therapy; LYM: lymphocyte count; PLT: platelet count; RTL: relative telomere length; SD: standard deviation; TFS: transplantation-free survival; MAA: moderate aplastic anemia; NEU: neutrophil count; NS: not significant; PLT: platelet count; PNH: paroxysmal nocturnal hemoglobinuria; RET: reticulocyte count; RTL: relative telomere length; SAA: severe aplastic anemia; SD: standard deviation; VSAA: very severe aplastic anemia; WBC: white blood cell count.

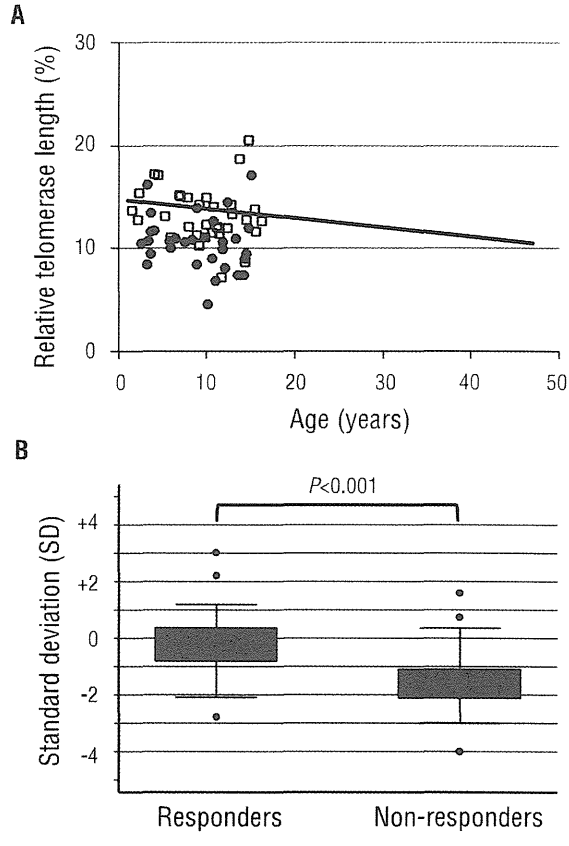

Figure 1. Relative telomere length in responders and non-responders. (A) Scatter plot of relative telomere length (RTL) *versus* age in patients with aplastic anemia (AA). The regression line for healthy individuals is shown as a solid line (Y= -0.0907X + 14.751). Results for AA patients were shown for responders (n=33; open squares) and non-responders (n=31; closed circles). (B) Comparison for telomere length between responders and non-responders. Box plots representing the distribution of telomere length in responders (n=33) and non-responders (n=31). The upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively; the horizontal bar across the box indicates the median and the ends of the vertical lines indicate the minimum and maximum data values. Dots indicate outliers.

responders (n=33), and -1.5SD (range -4.0 to +1.6SD) in non-responders (n=31) (Figure 1A and B). There was a significant difference in telomere length between responders and non-responders (P<0.001). We evaluated the effects of age-adjusted telomere length quartiles on the response rate. There was a significant relationship between hematologic response and telomere length. The response rates at six months were 12.5% in the first (the shortest), 37.5% in the second, 75% in the third, and 81.3% in the fourth (the longest) quartiles of telomere length (Figure 2). The most powerful cut-off point for dividing responders and non-responders by telomere length was -1.0 SD (P=6.9x10^{-6}). There was no statistical tendency between relapse rate / clonal evolution / overall survival and telomere length.

We evaluated the pre-treatment variables for predicting response to IST in 64 children with AA (Table 1). Univariate analysis showed that interval from diagnosis to IST longer than 25 days (P=0.01), platelet count at diagnosis less than 25x10^9/L (P=0.01), and telomere length shorter than -1SD

(P<0.001) were the variables statistically significant for poor response to IST, while there were no significant differences between responders and non-responders in terms of patient age, sex, disease severity, WBC count, neutrophil count, lymphocyte count, reticulocyte count, presence of HLA-DR15, and presence of minor PNH clones. Patients with rabbit ATG showed a tendency of poorer response to IST than patients with horse ATG (P=0.08).

Multivariate analysis confirmed that telomere length shorter than -1.0SD (HR 22.0; 95%CI: 4.19-115; P<0.001), platelet count at diagnosis less than 25x10⁹/L (HR 13.9; 95%CI: 2.00-96.1; P=0.008), and interval from diagnosis to IST longer than 25 days (HR 4.81; 95%CI: 1.15-20.1; P=0.031) were the significant predictive variables for poor response to IST (Table 2).

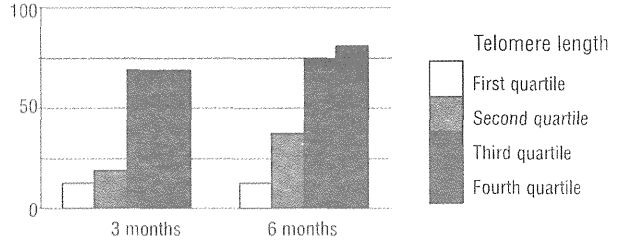

Figure 2. Response rates for immune suppressive therapy at three and six months according to telomere length. A poorer response rate was observed with each quartile as the telomere length shortened from the fourth to the first quartile.

Discussion

Our study demonstrated that the measurement of telomere length of lymphocytes is useful for predicting the response to IST in patients with AA. Recently, the NIH group reported that the telomere length of peripheral blood leukocytes was not related to hematologic response to IST, but was associated with the high risk of hematologic relapse, clonal evolution to myelodysplastic syndrome, and OS.[35] Several reasons may explain the conflicting results of the two studies. To begin with, there are several differences between the current study and the NIH study, including the methods of telomere length measurement and patients' characteristics. In the NIH study, the telomere length of pre-treatment total leukocytes was assessed by quantitative polymerase chain reaction (PCR). We measured the telomere length of lymphocytes using flow-FISH, which enabled us to measure median telomere length in the subpopulations of blood cells. Alter *et al.* compared the diagnostic sensitivity and specificity of short telomeres in different subpopulations of blood cells.[29] Their results indicated that lymphocytes were more suitable for diagnosis of DC than total leukocytes, which were a heterogeneous mixture of cell populations. The proportions of each cell population were different in each patient. The use of total leukocytes is suspected to provide less consistent results than analyses of defined leukocyte subpopulations.

Another difference between the two studies was the distribution of patients' age. Patients in our study were much younger (mean age 10 years) than those in the NIH study (mean age 35 years). Because telomeres shorten with age,[30] the differences in telomere length between patients and healthy individuals may become smaller in adults than in children. Moreover, in the NIH study, the cohort was restricted to patients with severe AA, and patients with moderate AA were not included. In contrast, 20 of 64 AA patients in our study had moderate disease. We could not estimate the frequency of clonal evolution since in our cohort there was only one patient who evolved into myelodysplastic syndrome during the observation period.

The causes of the difference in telomere length between responders and non-responders remain unknown. The short telomere length in non-responders may be ascribed to the presence of cryptic forms of IBMFS in the study cohort. Alter *et al.* reported that nearly all of the patients with both typical and cryptic DC have very short telomeres, as low as the first percentile of normal controls.[28] In our previous study, the RTLs of lymphocytes were below the 5% of normal controls in all of 6 DC patients and 2 AA patients harboring TERT mutation.[31] In the current study, there were 10 AA patients with shorter telomere length than the -2.0 SD of the cohort of healthy controls, but none of them showed clinical features of DC or had any mutation in *DKC1, TERC, TERT, NOP10, TINF2,* and *TCAB1*. It is unlikely that short telomeres in non-responders are to be ascribed to the presence of a cryptic form of DC.

Another possibility is that short telomere length may be a surrogate marker for longer disease duration that damages the hematopoietic stem cells and causes a higher number of compensatory stem cell divisions. We recently reported a significant inverse correlation between response rate to IST and interval between diagnosis and treatment in a large cohort of 312 children with newly diagnosed AA.[32] It is often difficult to determine the exact date of onset of the disease in patients with AA, especially in patients with moderate AA. The shorter telomere length may simply reflect longer duration of the disease in non-responders.

However, our study has several limitations, including a heterogeneous study population, a relatively small number of patients and a short follow-up period. To validate the results, we are conducting a prospective study to determine the optimal use of rabbit ATG for severe AA, in which we evaluate the relationship between telomere length of lymphocytes at diagnosis and the response to IST.

In conclusion, measurement of the telomere length in lymphocytes by flow-FISH is a promising assay, not only for identifying cryptic DC, but also for predicting the response to IST of patients with AA.

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Authorship and Disclosures

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References

1. Kojima S, Horibe K, Inaba J, Yoshimi A, Takahashi Y, Kudo K, et al. Long-term outcome of acquired aplastic anaemia in children: comparison between immunosuppressive therapy and bone marrow transplantation. Br J Haematol. 2000;111(1):321- 8.
2. Young NS. Acquired aplastic anemia. JAMA. 1999;282(3):271-8.
3. Kojima S, Hibi S, Kosaka Y, Yamamoto M, Tsuchida M, Mugishima H, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood. 2000;96(6):2049-54.
4. Fuhrer M, Rampf U, Baumann I, Faldum A, Niemeyer C, Janka-Schaub G, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. Blood. 2005;106 (6):2102-4.
5. Locasciulli A, Oneto R, Bacigalupo A, Socie G, Korthof E, Bekassy A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). Haematologica. 2007;92(1):11-8.
6. Nakao S, Takamatsu H, Chuhjo T, Ueda M, Shiobara S, Matsuda T, et al. Identification of a specific HLA class II haplotype strongly associated with susceptibility to cyclosporine-dependent aplastic anemia. Blood. 1994;84(12):4257-61.
7. Maciejewski JP, Follmann D, Nakamura R, Saunthararajah Y, Rivera CE, Simonis T, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and the PNH/aplastic anemia syndrome. Blood. 2001;98(13):3513-9.
8. Oguz FS, Yalman N, Diler AS, Oguz R, Anak S, Dorak MT. HLA-DRB1*15 and pediatric aplastic anemia. Haematologica. 2002;87(7): 772-4.
9. Sugimori C, Chuhjo T, Feng X, Yamazaki H, Takami A, Teramura M, et al. Minor population of CD55-CD59- blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. Blood 2006;107:1308-14.
10. Yoshida N, Yagasaki H, Takahashi Y, Yamamoto T, Liang J, Wang Y, et al. Clinical impact of HLA-DR15, a minor population of paroxysmal nocturnal haemoglobinuria-type cells, and an aplastic anaemia-associated autoantibody in children with acquired aplastic anaemia. Br J Haematol. 2008;142 (3):427-35.
11. Scheinberg P, Wu CO, Nunez O, Young NS. Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. Br J Haematol. 2009;144(2):206-16.
12. Walne AJ, Dokal I. Advances in the understanding of dyskeratosis congenita. Br J Haematol. 2009;145:164-72.
13. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell. 1985;43(2 Pt 1):405-13.
14. Szostak JW, Blackburn EH. Cloning yeast telomeres on linear plasmid vectors. Cell. 1982;29(1):245-55.
15. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature. 1999;402:551-5.
16. Calado RT, Young NS. Telomere diseases. N Engl J Med. 2009;361:2353-65.
17. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352(14):1413-24.
18. Vulliamy TJ, Marrone A, Knight SW, Walne A, Mason PJ, Dokal I. Mutations in dyskeratosis congenita: their impact on telomere length and the diversity of clinical presentation. Blood. 2006;107:2680-5.
19. Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC, Gordon-Smith EC. Progressive telomere shortening in aplastic anemia. Blood. 1998;91(10):3582-92.
20. Brummendorf TH, Maciejewski JP, Mak J, Young NS, Lansdorp PM. Telomere length in leukocyte subpopulations of patients with aplastic anemia. Blood. 2001;97(4):895-900.
21. Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, Young NS. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood. 2003;102 (3):916-8.
22. Leteurtre F, Li X, Guardiola P, Le Roux G, Sergère JC, Richard P, et al. Accelerated telomere shortening and telomerase activation in Fanconi's anaemia. Br J Haematol. 1999;105(4):883-93.
23. Pavesi E, Avondo F, Aspesi A, Quarello P, Rocci A, Vimercati C, et al. Analysis of telomeres in peripheral blood cells from patients with bone marrow failure. Pediatr Blood Cancer. 2009;53(3):411-6.
24. Thornley I, Dror Y, Sung L, Wynn RF, Freedman MH. Abnormal telomere shortening in leucocytes of children with Shwachman-Diamond syndrome. Br J Haematol. 2002;117(1):189-92.
25. Scheinberg P, Cooper JN, Sloand EM, Wu CO, Calado RT, Young NS. Association of Telomere Length of Peripheral Blood Leukocytes With Hematopoietic Relapse, Malignant Transformation, and Survival in Severe Aplastic Anemia. JAMA. 2010;304 (12):1358-64.
26. Kosaka Y, Yagasaki H, Sano K, Kobayashi R, Ayukawa H, Kaneko T, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood. 2008;111:1054-9.
27. Takahashi Y, Muramatsu H, Sakata N, Hyakuna N, Hamamoto K, Kobayashi R, et al. Rabbit antithymocyte globulin and cyclosporine as first-line therapy for children with acquired aplastic anemia. Blood. 2013;121:862-3.
28. Baerlocher GM, Vulto I, de Jong G, Lansdorp PM. Flow cytometry and FISH to measure the average length of telomeres (flow FISH). Nat Protoc. 2006;1(5):2365-76.
29. Alter BP, Baerlocher GM, Savage SA, Chanock SJ, Weksler BB, Willner JP, et al. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. Blood. 2007;110(5):1439-47.
30. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990;345(6274):458-60.
31. Nishio N, Kojima S. Recent progress in dyskeratosis congenita. Int J Hematol. 2010;92(3):419-24.
32. Yoshida N, Yagasaki H, Hama A, Takahashi Y, Kosaka Y, Kobayashi R, et al. Predicting response to immunosuppressive therapy in childhood aplastic anemia. Haematologica. 2011;96(5):771-4.