Challenges in HLH transplant: Tricks to prevent menace of mixed chimerism

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Abbreviations	
Conv MA	conventional myeloablative
DLI	donor lymphocyte infusion
EFS	event-free survival
GVHD	graft versus host disease
HLH	hemophagocytic lymphohistiocytosis
HSCT	hematopoietic stem cell transplant
OS	overall survival
PBSCT	peripheral blood stem cell transplant
RIC	reduced-intensity conditioning
RTC	reduced-toxicity regimen
SOS	sinusoidal obstructive syndrome
TRM	transplant related mortality

Primary hemophagocytic lymphohistiocytosis (HLH), including familial HLH and some inherited immune deficiency syndromes, is a rare, life-threating disease. It is caused by mutations of several genes that impair lymphocytes' cytotoxic machinery. The mutations mostly generate defects in perforin- and granzyme-dependent pathway, resulting in the inability of NK cells and cytotoxic T lymphocytes to down-regulate the immune response. It is characterized by dysregulated hyperinflammatory response that results in hypercytokinemia. Hematopoietic stem cell transplantation (HSCT) is the only curative option for patients

with primary HLH. After the HLH 94-2004 studies conducted by the Histiocyte Society, the 5 year survival rate is improved by HSCT from about 50% to 66%.[1] HSCT is associated with high incidence of complications, such as infections, sinusoidal obstructive syndrome (SOS), respiratory complications and high transplant related mortality (TRM).

In spite of international collaboration, survival after HSCT has not changed significantly in the last two decades in primary HLH. Unavailability of matched donors, susceptibility to conditioning-related toxicities, and high frequency of mixed chimerism remain challenge for this hyperinflammatory immune-regulatory disorder. Debates about best preparative regimens are ongoing without resolution. Recently, the use of reduced-intensity conditioning (RIC) regimens has shown favorable outcomes and lower rate of acute complications when compared to conventional HSCT.[2] Prospective RIC national HCT trial for HLH/primary immunodeficiency resulted in low early mortality and 1-year overall survival (OS) of 80% (HLH only disease 82%), but 66.7% 18-month OS (HLH only disease 68%).[1] HLH disease can recur when donor chimerism declines to less than approximately 20%. RIC regimens need to be optimized to decrease the mixed chimerism. It is very interesting that there are two articles publishing in this issue to address this question in different angle.

In the article by Wustrau et al[3], a retrospective multicenter study in Germany and Austria describes 60 patients with primary HLH who received transplants between 2009-16. A multivariate logistic regression model was applied to analyze the five potential risk factors for substantial mixed chimerism including donor type, graft source, conditioning alkylating agent, condition serotherapy and remission status before conditioning. They found that the donor matching status, whether 10/10 or not, is the only factor with significant impact on the prevalence of substantial mixed chimerism (defined as while blood donor Chimerism equal or less than 25% and / or secondary cell therapy such as donor lymphocyte infusion (DLI), stem cell boost, or secondary HSCT). The article by Ali et al[4] in this issue describes different preparative regimen associated with mixed chimerism. Ali's group studied 36 HLH patients from single institution with 9 patients received reduced-toxicity regimen (RTC) conditioning with the combination of treosulfan, cyclophosphamide, fludarabine and thymoglobulin for allogeneic HSCT between 2015-19. They found that RTC cohort had the best compound event-free survival (EFS) (lack of relapse, graft failure, second transplant or additional donor cell infusions, or death) of 89% comparing 73% in conventional myeloablative (Conv MA) regimen and 42% in RIC regimen while kept similar 2-year OS of 89% comparing with 73% in Conv MA regimen and 83% with RIC regimen.[4] According to these two groups' results, 10/10 matching with RTC regimen can result in better outcome for HSCT in primary HLH.

Optimization of preparative regimen for HLH transplant is the key to get better donor chimerism and avoid secondary intervention (See summary in table). Serotherapy is a crucial factor for patient's survival and the development of mixed chimerism. Serotherapy is definitely needed for transplant in this hyperinflammatory disease as shown in Slatter's study (50% early mortality).[5] Willemsen et al[6] have shown that alemtuzumab was more likely to result in long-term mixed chimerism than ATG. Interestingly, in multivariate analysis serotherapy agent was not associated with mixed chimerism in Wustrau et al's article in this issue[3]. The timing and dosing of alemtuzumab greatly affect HLH patients after HSCT whether they develop mixed chimerism or GVHD. Both proximal administration and distal schedule with alemtuzumab more than 2mg/kg have been shown with increased mixed chimerism with 53% patients needing more secondary stem cell intervention in Marsh et al's study.[7] Intermediate alemtuzumab schedule with 1mg/kg divided by 5 days started on day -14 combined with fludarabine and melphalan generated better results with less mixed chimerism.[7] Ali's article concluded that ATG, combined with treosulfan and second alkylator cyclophosphamide in their RTC cohort, had the best result with 2-year OS and EFS of 89%.[4] In both articles in this issue, treosulfan combined with other alkylator cyclophosphamide/thiotepa yielded the best result with least mixed chimerism. Treosulfan is currently available in Europe – Asia but not in US. Wustrau et al noted that treosulfan versus melphalan did not change the incidence of mixed chimerism but adding thiotepa decreased that incidence from 50% to 25%. Similar observation was made in recently published article by Naik et al.[8]How active disease status affects patients survival is not widely studied. As the new IFN-γ targeting therapy-Emapalumab is approved for primary HLH treatment, it is encouraging that more patients will pro-

ceed to HSCT in remission. Vallurupalli and colleague's study showed that 65% of the relapsed /refractory HLH patients had overall remission and proceeded to HSCT with 90.9% post-HSCT survival.[9] The recent phase 2-3 study confirmed that 65% of total of 26 primary HLH patients had response in 8-week treatment period of Emapalumab and 70% were able to proceed to transplant with 89.5% estimated 12 months' survival.[10]

It is suggested that in primary immunodeficiency disorders, peripheral blood stem cell transplant (PBSCT) can ensure sustained high-level donor chimerism in more than 90% patients.[11] It is reasonable to think about PBSCT as a better stem cell source. These two articles in current issue provide food for thought to conduct prospective multicenter trial including melphalan/treosulfan with cyclophosphamide/thiotepa with ATG as serotherapy may be with PBSCT as stem cell source.

Table. Summary of different conditions and their outcomes in the transplant of HLH [a].

Literature (Patient#)	Marsh et al 2013[7] (79)	Allen et al 2018[1] (34)	Furtado-Silva et al 2019[12] (118)	Ali et al 2020[4] (36)	Wustrau et al 2020[3] (60)
Serotherapy	Campath	Campath	ATG/Campath: 86%	ATG 67%; Campath 33%	ATG/thymoglobulin 33%; Campath 67%
Alkylating agent	RIC[f] (melphalan)	RIC (Melphalan)	MAC [f] (busulfan/Treosulfan) 90%; RIC (Melphalan) 10%	MAC(Busulfan/CPM) 42%; RTC(Treosulfan/CPM) 25%; RIC(Melphalan) 33%	Treosulfan/Thiotepa 75%; Melphalan/Thiotepa 27%
Stem cell source [b]	BM 96%; PBSC 4%	BM	Cord	BM 69%; PBSC 14%; Cord 17%	BM 66%; PBSC 32%; Cord 2%
Donor [c]	Matched 75%; mismatch 25%. Sibling 24%; Unrelated 76%	MRD 21%; MUD 47%; MMUD 32%	HLA-Match 6/6:30% HLA-Mis 5/6: 48% HLA-Mis [?]4/6: 22%	MRD 25%; MMRD 8%; MUD 50%; MMUD 17%	MRD 17%; MUD 37%; MMRD 10%; MURD 37%
2nd cell therapy [d]	19%	35%	–	RTC 0%; MAC 13%; RIC 67%	32 %
EFS [e]	– [g]	–	6 yr: 34˜69%	2 yr: RTC 89%; MAC 73%; RIC 42%	–
OS	1 yr: 80˜91% 5 yr: 80˜82%	1 yr: 82%; 18-month: 68%	6 yr: 55%	2 yr: RTC 89%; MAC 73%; RIC 83%	5 yr: 75%

a Numbers in the first row within parentheses are sample numbers

b Stem cell source: BM, bone marrow; PBSC, peripheral blood stem cell; Cord, cord blood

c Donor: MRD, matched related donor; MUD, matched unrelated donor; MMRD, mismatched related donor; MMUD, mismatched unrelated donor

d 2nd cell therapy: donor lymphocyte infusion, stem cell boost or HSCT.

e EFS, event-free survival; OS, overall survival

f MAC, busulfan based conventional myeloablative conditioning; RIC: melphalan based reduced intensity conditioning; RTC: treosulfan based reduced toxicity conditioning; CTX: cyclophosphamide.

g –, data not available.

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