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HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative approach to children and adolescents with high-risk acute lymphoblastic leukemia (ALL) at diagnosis or relapsed disease. Nonetheless, despite the graft versus leukemia (GVL) effect, treatment-related morbidity and mortality remains a major challenge. Moreover, the significant heterogeneity of the available data on the selection of patients, type of conditioning regimen, and type of donor hampers any definitive conclusions in the pediatric population. In 2020, the Brazilian Group for Pediatric Bone Marrow Transplantation of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy (SBTMO) and the Brazilian Society for Pediatric Oncology (SOBOPE) convened a task force to provide general guidance on HSCT for childhood ALL to providing evidence-based guidance for the appropriate management of this disease.

Keywords: Hematopoietic Stem Cell Transplantation;. Pediatric Acute Lymphoblastic Leukemia . Clinical Guidelines

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative approach to children and adolescents with high-risk acute lymphoblastic leukemia (ALL) at diagnosis or relapsed disease. Nonetheless, despite the graft versus leukemia (GVL) effect provided by this procedure, treatment-related morbidity and mortality remains a major challenge in this scenario. Moreover, the significant heterogeneity of the available data on the selection of patients, type of conditioning regimen, and type of donor hampers any definitive conclusions in the pediatric population (1).

In 2020, the Brazilian Group for Pediatric Bone Marrow Transplantation of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy (SBTMO) and the Brazilian Society for Pediatric Oncology (SOBOPE) convened a task force to review and update the main indications for HSCT for childhood ALL based on previous guidelines, with a view to providing evidence-based guidance for the appropriate management of this disease (Table 1).

TABLE 1: HSCT indications for pediatric ALL

HSCT indications for pediatric ALL in first remission:
ALL diagnosed before 6 months of age associated with MLL (KMT2A) rearrangement and with other risk factors, such as hyperleukocytosis (> 300,000/mm3) and non-response to corticosteroids.
Children who fail induction therapy (M2/M3 marrow), except if hyperdiploid ALL and age less than 6 years.
The current evidence does not support the use of HSCT in first remission for children with Ph+ (Bcr/Abl) ALL and hypodiploidy who have a good response to chemotherapy (CT).
HSCT is indicated for B- or T-cell ALL in first remission in patients with an MRD equal to or greater than 10-3, or 0.1%, by the end of the consolidation phase (i.e., after approximately 12 weeks of treatment)
HSCT indications for pediatric ALL in *second remission:*
Early bone marrow (BM) relapse of B-cell ALL (< 36 months after first remission). In late BM or extramedullary relapse of B-cell ALL, CT and HSCT exhibit similar results, so HSCT should be preferred, except in cases with persisting MRD positivity.
Early isolated extramedullary relapse of B-cell ALL (< 18 months of first remission).
Any, early or late, medullary, or extramedullary, relapse of T-cell ALL.

HSCT indications for pediatric ALL in *first remission*: Despite the different classification schemes and array of biologic and molecular risk factors recognized as highly relevant in the past, the advancements seen in the last few years have identified suboptimal response or persistence of minimal residual disease (MRD) after induction and consolidation therapy as the main risk factors indicating the benefit of HSCT [2,3], provided a minimal sensitivity of 10^{-4} (by analyzing a minimum of 1 million cells) technique and standardized protocol are used.

Of note, the choice, suitability, and definitions of the protocol to be used in the first-line treatment of childhood ALL are key when considering referral for transplantation in first remission. Indications not guided by defined protocols are:

HSCT is indicated for B- or T-cell ALL in first remission in patients with an MRD equal to or greater than 10^{-3}, or 0.1%, by the end of the consolidation phase (i.e., after approximately 12 weeks of treatment) [2,4,5,6].

HSCT in first remission for infants with ALL diagnosed before 6 months of age associated with *MLL (KMT2A) rearrangement* and with other risk factors, such as hyperleukocytosis (> 300,000/mm^3) and non-response to corticosteroids (7).

HSCT in first remission is indicated for children who fail induction therapy (M2/M3 marrow), except if hyperdiploid ALL and age less than 6 years (1,8,9).

The current evidence does *not* support the use of HSCT in first remission for children with Ph+ (*Bcr/Abl*) ALL and hypodiploidy who have a good response to chemotherapy (CT) [9, 10, 11].

HSCT indications for pediatric ALL in *second remission*: Early bone marrow (BM) relapse of B-cell ALL (< 36 months after first remission). In late BM or extramedullary relapse of B-cell ALL, CT and HSCT exhibit similar results, so CT should be preferred, except in cases with persisting MRD positivity [12, 13].

Early isolated extramedullary relapse of B-cell ALL (< 18 months of first remission).

Any, early or late, medullary, or extramedullary, relapse of T-cell ALL (13).

In third remission: From third remission onwards, survival at 5 years after HSCT varies between 26 and 33%, as compared to 15% after CT. Patients without morphological remission do *not* benefit from transplantation [14].

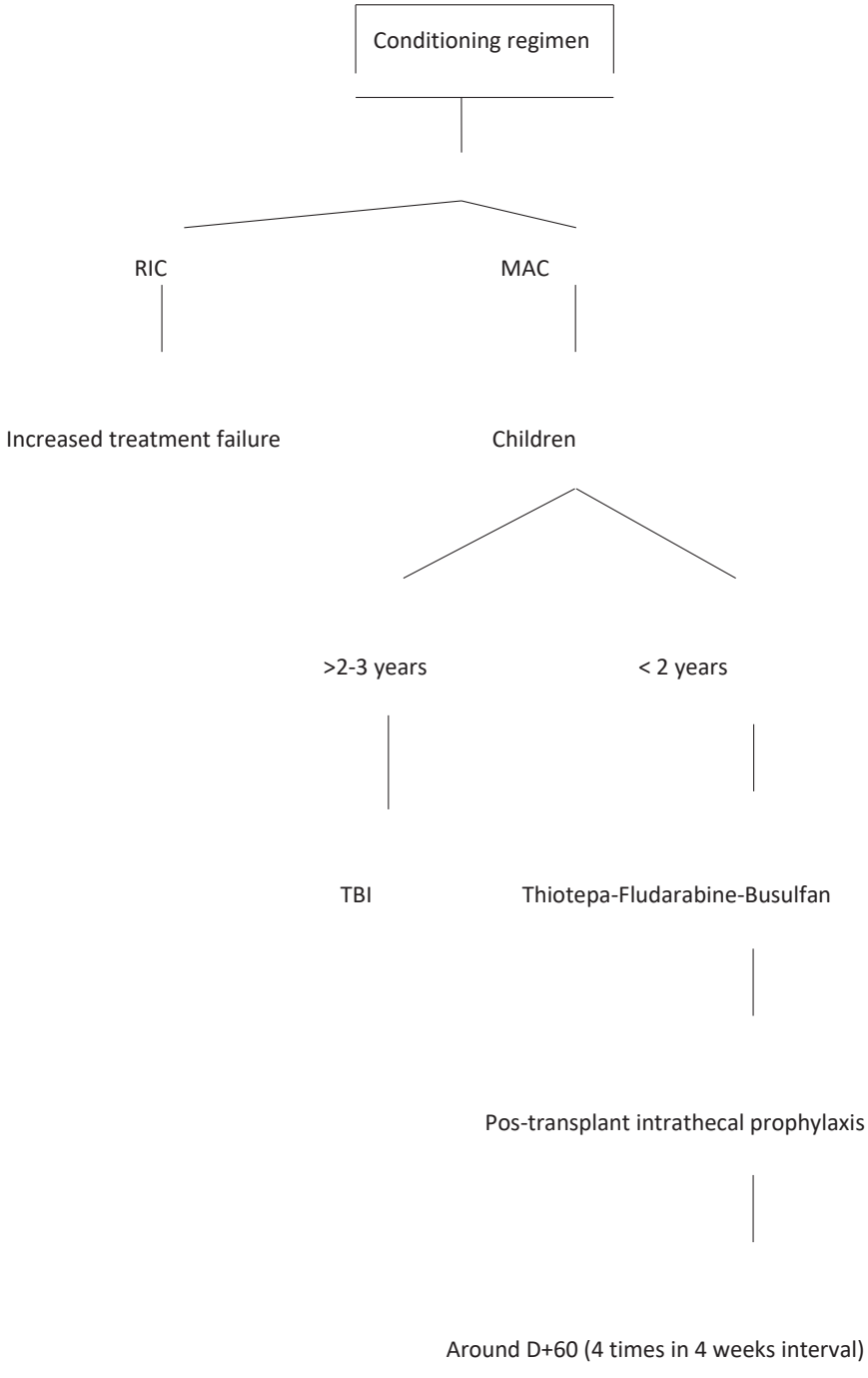
WHICH IS THE BEST DONOR AND STEM-CELL SOURCE?

Despite the better overall survival (OS) and mortality results seen with HLA-matched sibling donor transplants, there is current evidence that unrelated donors with a greater than 8/10 HLA-match and haploidentical donors provide fairly similar results [15, 16, 17]. In children, bone marrow is preferable in comparison to peripheral blood (PB) as stem-cell source, given the higher extensive chronic GVHD and transplant-related mortality (TRM) with the use of PB stem cells [8, 18]. The use of Umbilical Cord Blood (UCB) is associated with higher TRM in Brazil and should only be used by centers experienced with this stem cell source [19].

WHICH IS THE BEST CONDITIONING REGIMEN?

Myeloablative conditioning (MAC) regimens remain the standard of care for HSCT in childhood ALL. Reduced intensity conditioning (RIC) has not been shown to be of benefit in the treatment of ALL due to increased treatment failure (Figure 1) [20].

FIGURE 1. Flowchart of conditioning choice for Pediatric Acute Lymphoblastic Leukemia



Even though most children with ALL undergo HSCT with myeloablative conditioning regimens including total body irradiation (TBI), recent studies are trying different CT-based protocols to effectively replace TBI, particularly in children under 2 years of age. However, a retrospective study comparing TBI versus CT showed that TBI-based conditioning has better outcomes (OS and non-relapse mortality) and is the standard of care in the treatment of ALL in children older than 2-3 years old [21]. The only prospective trial randomizing children older than 4 years to conditioning therapy with TBI - Etoposide or Thiotepa - Fludarabine - Busulfan (or Treosulfan) demonstrated superiority of TBI in terms of lower relapse rate, TRM, and improved OS (91% vs. 75%, p<0.0001) [22].

Central nervous system (CNS) boost irradiation in the context of TBI is less commonly indicated but could be useful in certain scenarios (CNS involvement at diagnosis or at relapse) for treating and preventing CNS relapse after allo-HSCT [22,23].

The International "Forum" protocol also recommends post-transplant intrathecal prophylaxis whenever TBI is not part of the conditioning therapy. They suggest four weekly triple intrathecal administrations starting around D+60 if the patient is already stable and with greater than 50.000 platelets/mm3[22].

TBI has historically been used in combination with high doses of cyclophosphamide (120mg/kg), with favorable OS and event-free survival (EFS) results, yet considerable short- and long-term toxicity. Over the past few years, the association of TBI with etoposide (60mg/kg) has yielded somewhat better results in respect to OS, disease-free survival (DFS), and TRM [22]. The incorporation of other drugs to the preparative regimen, such as thiotepa, fludarabine, and melphalan would need further studies.

WHAT IS THE BEST GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS REGIMEN IN CHILDHOOD ALL?

In HLA-matched sibling donor (MSD) allo-HSCT, calcineurin inhibitors (Cyclosporine – CSP 3 mg/kg or Tacrolimus – TAC 0.05mg/kg in two divided I.V. doses a day) as a single agent should be started on D-1, and switched to their corresponding oral formulations, with strict dose adjustment based on serum levels (100-200mcg/L for CSP - or 80 and 130 ng/mL if the methods measure CSP without its metabolites as fluorescence polarization immunoassay (FPIA) and enzyme-multiplied immunoassay technique (EMIT)- and 5-15ng/ml for TAC), until 3 months after transplant, with subsequent tapering, in the absence of graft-versus-host disease (GVHD)(24,25,26,27). In HLA-matched unrelated donor (MUD) HSCT, prophylaxis with short-term methotrexate (MTX) combined with either CSP or TAC showed similar results. The use of single-agent, post-transplant cyclophosphamide (PTCy) at a dose of 50mg/kg for two days on D+3 and D+4 (or D+5) has shown similar results regarding GVHD control, although further studies are awaited to define the optimal regimen in terms of long-term outcome for these patients (Table 2) [28,29, 30].

TABLE 2. GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS REGIMEN IN CHILDHOOD ALL

MSD	CSP 2mg/kg or TAC 0.05mg/kg in two divided IV doses– started on D-1 (SL CSP: 100-200mcg/L or TAC: 5-15 ng/ml)
MUD	Short-term MTX (D+1, D+3, D+6)* + CSP or TAC
HAPLO	PTCy 50mg/kg (D+3 and D+4)** + CSP or TAC + MMF 15mg/kg/dose q8h; max 2g/day – started on D+5
UCB	Combination of CSP or TAC + MMF 15mg/kg/dose q8h; max 2g/day

*MTX is used at doses of 10mg/m2, all of which with leucovorin rescue after 24h
**coupled with mesna (100-160% of the Cy dose)
MSD, matched sibling donor; CSP, cyclosporin; TAC, tacrolimus; SL, serum levels; MTX, methotrexate; MUD, matched unrelated donor, HAPLO, haploidentical; PTCy, post-transplant cyclophosphamide; MMF, mycophenolate mofetil; UCB, umbilical cord blood

In unrelated allo-HSCT, CSP (at the same dose as that for related donor transplants) combined with MTX for a short period of time (i.e, on days +1, +3, +6 and +11) is the standard prophylactic regimen. MTX is used at an initial dose of 15mg/m^2 at most centers, followed by three doses of 10mg/m^2, all of which with leucovorin rescue after 24h of each dose for the prevention of oral mucositis. TAC at a total daily dose of 0.05mg/kg can also be used, with similar results. In contrast, the combination of mycophenolate mofetil (MMF) with CSP was shown to be less effective [31]. Although the use of anti-thymocyte globulin (ATG), primarily for the prevention of GVHD, has been consolidated in unrelated donor HSCT in adults, there is limited evidence as to its benefit in the pediatric population, even though it is used in most protocols. In a randomized study comparing different dose regimens of ATG, use of ATG at lower doses was associated with a reduction in the rate of infection while maintaining similar acute and chronic GVHD rates, as well as relapse rates. The investigators concluded that low-dose ATG should be the standard serotherapy regimen for URD HCST in children with hematologic malignancies [32], even though it should be borne in mind that the different ATG formulations available have variable immune responses, which may hinder any definitive conclusions as to its real benefit in this regard.

In haploidentical HSCT, cyclophosphamide is generally used at a dose of 50mg/kg/day, in a 2-hour infusion, on D+3 and D+4, coupled with mesna (100-160% of the cyclophosphamide dose), in combination with a calcineurin inhibitor (CSP or TAC) and MMF (15mg/kg/dose q8h; maximum dose 2g/day), both starting on D+5. Both these immunosuppressants are usually kept for 3 months post-transplant [33,34].

As for UCB transplantation, the immunosuppressive regimen usually comprises the combination of a calcineurin inhibitor with MMF. Studies on the association of CSP with low-dose MTX or with corticosteroids have yielded worse results, as well as a greater rate of graft failure [28].

CLINICAL SIGNIFICANCE OF MRD FOR HSCT IN ALL

Persistence of MRD positivity at the end of consolidation therapy has been associated with a high risk of relapse and the need for intensification of therapy [2, 6, 35, 36]. Patients with MRD ≥ 10-3 (0.1%) at this time point of treatment can benefit from allo-HSCT in first remission [2, 3].

Children and adolescents with high-risk relapsed ALL are eligible for allo-HSCT in second remission (CR2) or over [25]. In children with relapsed or high-risk ALL, MRD ≥10– 3 before HSCT indicates a highly resistant disease to conventional intensive CT. These patients are candidates for new therapeutic strategies, including targeted- or immunotherapy, to reduce the tumor burden and the risk of post-transplant relapse [3, 13].

Levels of MRD pre- and post-allo-HSCT have been shown to have a prognostic impact: patients with undetectable MRD before MAC allo-HSCT have a better outcome than those with any level of MRD positivity [3, 13, 35, 36,37,38,39]. In these series of patients, the discriminatory detection limits of MRD were defined as 10-3 and 10-4 [13, 36, 40]. Bader et al., 2009, showed that patients with pre-transplant MRD < 10-4 (0.01%) had a higher EFS and a lower cumulative incidence of relapse (CIR) than those having undergone allo-HSCT with MRD ≥ 10-4 [37].

Persistence of MRD positivity after transplantation is related to significantly worse outcomes compared to patients with undetectable MRD, regardless of the method used for MRD detection [3, 35,38-41]. On the other hand, conversion of an MRD-positive status into a negative one after transplant is associated with longer remission and lower relapse risk [3,35]. This has also been observed in the haploidentical HSCT scenario [43].

The prognostic utility of pre- and post-transplant MRD kinetics has been demonstrated as follows: (i) patients with detectable pre- and post-HSCT MRD, particularly those with higher MRD levels (≥ 0.1%), have significantly lower EFS and higher CIR; (ii) lower levels of pre-HSCT MRD (<10-4) converting into undetectable post-HSCT MRD do not have a negative impact on outcome; (iii) even low levels of post-HSCT MRD are invariably correlated with a higher risk of relapse ($p = 0.001$) [3]. In short, the risk of relapse is more strongly influenced by post-transplant MRD than by pre-transplant MRD [3]. Close surveillance and preemptive immunotherapy strategies post-transplant have been shown to effectively decrease the relapse rate in the high-risk population [44, 45].

BEST TIME POINTS FOR MRD ASSESSMENT:

Pre-HSCT: MRD assessments should be made immediately before allo-HSCT [13]. Berlin-Frankfurt-Munich (BFM) study protocols recommend an MRD assessment to be made at a median of 13 days before allo-HSCT to verify the prognostic significance of MRD prior to transplantation [37]

Post-HSCT: MRD assessments by multiparameter flow cytometry (MFC) and/or reverse transcription quantitative polymerase chain reaction (RT-qPCR)

are accurate in predicting relapse at days +30, +60, +90, and +180 post-HSCT. From D+60 onwards, the discriminatory power of MRD detection was shown to be greater in predicting the probability of relapse [39]. However, using a more sensitive method to detect MRD, such as next generation sequencing (NGS), even earlier time points after transplant (i.e., at D+30) are also predictive of relapse ($p < 0.0001$) [42].

Any detectable MRD level on days +180 and +365 post-HSCT is highly predictive of relapse and poor survival. On the other hand, negative MRD on D+365 is associated with long-term survival [3]. Several factors can impact the outcome of pediatric patients with ALL undergoing allo-HSCT, such as: peri-transplant MRD positivity, remission status (CR2, CR3), non-TBI conditioning regimen, and absence of acute GVHD by D+190 post-transplant. These factors can define subgroups of children who are at a higher risk of relapse and who may thus benefit from successive MRD assessments and early therapeutic interventions [3]

It is very important to note that most studies determine MRD with very specific real-time qPCR of immunoglobulin and TCR gene rearrangements because the flow cytometric analysis of a reactive pediatric marrow can be extremely challenging. When decisions that may potentially change patient management are based on low levels of MRD, we would recommend that the SBTMO – MRD Working Group review the flow cytometric data to increase accuracy of the results.

CONSIDERATIONS ON ALL SPECIFIC GENETIC SUBGROUPS

Several biologic characteristics in ALL patients are significantly associated with MRD status during treatment [46]. Patients with good-risk cytogenetics (ETV6-RUNX1, high hyperdiploidy) demonstrate faster clearance of leukemic cells (MRD < 1x10-5), while patients with high-risk features (iAMP21, KMT2A rearrangement, haploidy/ hypodiploidy) respond more slowly [47,48]. Intermediate-risk cytogenetics, such as TCF3-PBX1 or t(1;19), have variable MRD kinetics: even though they exhibit faster disease clearance, such patients need more intensive therapy to avoid relapse [48,49]. Children with B-cell precursor (BCP) ALL with other genetic abnormalities, including alterations in copy number, BCR-ABL1-like mutations, JAK-STAT abnormalities, IKZF1 deletion, and IKZF plus usually exhibit prolonged MRD persistence [48-51].

Intrachromosomal amplification of chromosome 21 (iAMP21) ALL is considered a high-risk disease which requires an intensive treatment approach [52,53]. The BFM group considers that MRD alone can identify iAMP21 as a high-risk cytogenetic feature in ALL patients [54].

Ph1 + ALL patients who reach an MRD level of ≤ 10-4 leukemic cells at the end of induction therapy have a lower risk of relapse and have been shown to achieve high survival rates without undergoing transplantation [55,56]. Conversely, persistence of MRD positivity at later time points of therapy in Ph1+ ALL patients is associated with a higher incidence of disease relapse [55].

T-cell ALL is also associated with MRD kinetics, with a slower blast clearance compared to BCP-ALL when delivered the same therapy. However, patients with MRD < 0.01% at the end of induction and consolidation therapy may harbor a favorable prognosis (57), whereas those with high MRD (≥ 0.1%) levels at the end of the consolidation phase tend to exhibit a high risk of relapse [57]. Early T-cell precursor (ETP)-ALL is also associated with high levels of MRD after induction therapy and lower long-term outcomes [58]. Intensification of therapy, based mainly on the high MRD status, has resulted in comparable outcomes in ETP-ALL and non-ETP-ALL in pediatric patients [59].

Although the risk of relapse is directly proportional to the level of MRD in each cytogenetic risk group, the absolute risk of relapse associated with a specific level of MRD varies according to the genetic subtype. Hence, the integration of genetic biomarkers and MRD testing may improve risk stratification algorithms for treatment decision in this population [47-49]. This seems particularly promising for peri-HSCT interventions, which may lead to a significant improvement in transplant outcomes for children with ALL [60].

In patients relapsing after first allogeneic transplant, therapeutic options may be a second allogeneic transplant in a subsequent remission, targeted immunotherapies, and palliative care [61]). In patients relapsing after haploidentical transplants, it is important to note that one third of the patients may have a patient haplotype loss in the leukemic cells, rendering the disease invisible to the patient's immune system but 100% incompatible with a graft from a family member with the other haplotype [62]. For these patients, a second haploidentical HSCT may be the ideal treatment strategy.

CONCLUSIONS

Allo-HSCT remains the treatment of choice for children with high-risk or relapsed ALL. Over the past few decades, the results seen with URD transplants

have progressively improved, with similar outcomes as those shown with matched sibling donors. The relatively recent advent of the PTCy platform in haploidentical transplantation has overcome the challenge of finding allogeneic compatible donors. Nonetheless, a number of factors ought to be taken into account to achieve a favorable outcome after allo-HSCT in childhood ALL, among which, the advantages and limitations of conditioning regimens containing TBI, the optimal GVHD prophylaxis regimen, and the long-term follow-up of this population.

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