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ACUTE AND CHRONIC GRAFT-VERSUS-HOST-DISEASE – A FOCUS ON PEDIATRIC PATIENTS

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ABSTRACT

Graft-versus-host disease (GVHD), either in its acute or chronic form, is the main contributory factor for morbidity and non-relapse mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Recent advancements in the classification of this disease, with better applicability and reproducibility of standardized criteria, coupled with improvements in the management of steroid-refractory or resistant cases, have led to promising results. In 2020, the Brazilian Group for Pediatric Bone Marrow Transplantation of the Brazilian Society for Blood and Marrow Transplantation and Cellular Therapy (SBTMO) convened a task force to provide updated, evidence-based guidance for the diagnosis, classification, staging, prophylaxis, and treatment of GVHD, with a focus on the pediatric population, the results of which are presented here.

Keywords: Graft-Versus-Host Disease, Diagnosis, Classification, Grading, Staging, Prophylaxis, Treatment, Hematopoietic Stem Cell Transplantation, Pediatric, Consensus Guidelines.

DEFINITION AND RISK FACTORS FOR ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE

The original classification of acute graft-versus-host disease (GVHD) was published in 1974 and was based on time of onset as the sole criterion. From 2005 onwards, patients presenting with typical acute GVHD (aGVHD) symptoms before D+100 were categorized as having "classical aGVHD", whereas those with such manifestations starting after D+100 were classified as having "late onset or recurrent aGVHD"[1].

In 2005, the *National Institutes of Health* (NIH) published a new set of consensus guidelines harboring both the diagnostic and the grading criteria for chronic GVHD (cGVHD), including various aspects pertaining to the diagnosis, classification, and treatment of this post-hematopoietic stem cell transplant (HSCT) complication[1,2].

Roughly a decade later, in 2014, the NIH updated these guidelines, which kept the original structure, but added more robust evidence-based guidance for the diagnosis and management of cGVHD[3]. These guidelines focused on controversial aspects, including, but not limited to, the distinction between active disease and prior tissue injury. Additionally, the diagnostic criteria for target-organ involvement, such as mouth, eyes, genitalia, and lungs, were thoroughly revised, and cGVHD-related organ impairment was specifically addressed. In short, this update aimed at a comprehensive diagnostic and prognostic assessment of cGVHD, as well as at better guidance toward appropriate treatment and defining eligibility for clinical trials, with greater specificity and precision.[3].

A number of risk factors have been widely recognized as related to an increased incidence of

aGVHD[4,5,6]. These factors may be directly related to the recipient, the donor, the graft, or the HSCT itself. Age, baseline disease, sex (particularly female donor to male recipient combinations), Human Leukocyte Antigen (HLA) mismatch, conditioning regimen intensity, GVHD prophylaxis used, stem cell source (peripheral blood > bone marrow > umbilical cord blood), CD34+ count, T-lymphocyte depletion, and infection risk are among the main risk factors in this regard.

During the last few years, several biomarkers have been investigated as potential surrogates for a greater occurrence of aGVHD or a worse response to therapy[7]. In this respect, a panel of four biomarkers has been more widely investigated: suppressor of tumorigenesis 2 (ST2), regenerating islet-derived 3-alpha (REG3α), tumor necrosis factor (TNF) alpha receptor type 1(TNFR1α) and interleukin-2 receptor alpha (IL-2Rα orCD25)[7]. ST2, for instance, has been shown to be an important biomarker of treatment-resistant aGVHD[7]. Nonetheless, these biomarkers are not yet available for use in clinical practice in Brazil.

The Endothelial Activation and Stress Index (EASIX) serves as a practical tool for identifying patients with high-risk GVHD, since it is based on readily available laboratory markers, namely: lactate dehydrogenase (LDH), serum creatinine, and platelet count[8]. The EASIX score may be useful for identifying patients, including children, with aGVHD who are at greater risk of death, particularly in the reduced-intensity conditioning (RIC) setting, where a statistically significant difference was shown[8]. The EASIX score may thus become an important clinical tool for the development of a risk-adapted strategy toward the treatment of GVHD[8].

As for cGVHD, the main underlying risk factor is a prior history of aGVHD.

ACUTE GVHD DIAGNOSIS AND CLINICAL DESCRIPTION

Acute GVHD is a reaction of donor immune cells against host tissues which can occur after allogeneic HSCT (allo-HSCT). The three main tissues affected by acute GVHD are skin, liver, and gastrointestinal (GI) tract. Its onset normally correlates with engraftment of donor cells.

Acute GVHD is commonly suspected based on the clinical presentation that represents the organs involved. The earliest and most common manifestation is skin GVHD. This is essentially a maculopapular rash that can begin anywhere in the body but often starts in palm and sole, with or without pruritus or tenderness in affected areas. If the rash progresses, it may become confluent. In severe cases, blisters may occur. The GI manifestations include diarrhea, which may become bloody, cramping, nausea, and vomiting. Furthermore, jaundice from hyperbilirubinemia is the hallmark of liver GVHD[9], although a hepatitic variant of GVHD with elevated liver enzymes, as in an acute viral hepatitis, has been recognized[10]).

The diagnosis of aGVHD is a clinical one but, as many of the symptoms of aGVHD are non-specific, histologic confirmation, whenever possible, may be extremely useful. Tissue biopsy is recommended to confirm a histological diagnosis of aGVHD and, most importantly, to exclude opportunistic infection or drug toxicity. However, the combination of rash, nausea, and diarrhea, occurring after neutrophil engraftment renders the diagnosis very likely. The histologic hallmark of cellular injury by GVHD is apoptosis, which is observed in basal epidermal keratinocytes, bile ducts, and/or intestinal crypt epithelial cells and is frequently associated with lymphocyte infiltration[11].

GRADING OF AGVHD

As mentioned above, the skin, GI tract and liver are the main target organs affected in aGVHD. The first organ affected is most often the skin, which is clinically manifested as a maculopapular rash in the nape, cheeks, ears, shoulder (head end), palms and soles. It can disseminate throughout the body surface (BS) and become confluent and, sometimes, itchy. In severe forms, bullous wounds secondary to epidermal necrosis occur. The degree of cutaneous involvement is quantified by the extent and severity of lesions, as described in tables 1 and 2.

Regarding the GI tract, it often affects both its upper and lower portions. It may clinically present with anorexia, nausea, vomiting, diarrhea, and abdominal pain. The diagnosis can be confirmed by histopathological examination of biopsies obtained through upper digestive tract endoscopy, rectal biopsy or, in some cases, colonoscopy, depending on the risk of bleeding. Several studies, including a recent prospective one, suggest that most GI tract GVHD diagnoses can be made through rectal biopsies[12].

It is important to note that a negative rectal biopsy does not rule out aGVHD, for which further endoscopy is required to confirm the diagnosis and differentiate it from other common pathologies, mainly infections, of the early post-HSCT period.

The degree of GI tract involvement is classified by the severity of the diarrhea, as described in tables 1 and 2.

GVHD of the *lower* GI tract is usually severe, with or without hematochezia and abdominal cramps. The diarrhea is often watery and abundant (up to several liters per day) and may become bloody. In such cases, it is of utmost importance that blood transfusion support is assured, along with hydroelectrolytic replacement, use of opiates to control pain, and close monitoring due to the risk of hemodynamic instability.

As diarrhea is a common presentation in the immediate post-HSCT period and can be caused by organic toxicity due to the conditioning regimen or by broad-spectrum antibiotics, histopathological examination may serve as a useful diagnostic tool to exclude bacterial toxins or concomitant cytomegalovirus (CMV) infection.

GVHD of the *upper* GI tract must also be differentiated from herpes simplex virus infection, candida esophagitis, peptic ulcer, and secondary toxicity, which can be achieved by endoscopy.

The liver injury caused by GVHD generally occurs in patients with concurrent signs of skin and / or GI tract GVHD and is only rarely seen in its isolated form. It often presents itself with abnormal liver function tests, with a characteristic rise in total bilirubin (predominantly in its conjugated form) and alkaline phosphatase. It can progress to painful hepatomegaly, fluid retention, and pruritus. In a few cases, coagulopathy may be present.

These laboratory abnormalities reflect biliary canaliculi destruction, leading to cholestasis. However, these changes are non-specific and should be differentiated from those of other disorders, such as sinusoidal obstruction syndrome (SOS), viral hepatitis, and drug toxicities (from conditioning, antimicrobial therapy, or GVHD prophylaxis). Liver biopsy may play an important role in the diagnosis, but it is generally not feasible due to the high risk of bleeding.

The graduation of hepatic GVHD is based on bilirubin serum levels and is also summarized in tables 1 and 2, below.

The most popular systems for graduating GVHD are those of Glucksberg (grades I-IV) and the *International Bone Marrow Transplant Registry* (IBMTR)(A-D)[13,14]. The severity of aGVHD is determined by assessment of the degree and extent of each organ involved, as summarized in tables 1 and 2. The stages of individual organ involvement are combined using the Glucksberg with or without the *IBMTR criteria*. Grade I(A) aGVHD is characterized as mild disease, grade II(B) as moderate, grade III(C) as severe, and grade IV(D), as life-threatening[14,15].) The IBMTR grading system defines the severity of aGVHD as follows (adapted for children from Rowlings PA, 1997 and Carpenter PA, 2010)[13,11,16]:

- Grade A – Stage 1 skin involvement alone (rash of <25% of BSA with no liver or GI involvement);
- Grade B – Stage 2 skin involvement; Stage 1 to 2 gut or liver involvement (rash of 25-50% of BSA; diarrhea 10-19.9ml/kg/day – stage 1; diarrhea 20-30ml/kg/day - stage 2; bilirubin 2.1 to 3.0 mg/dL – stage 1; bilirubin 3.1 to 6.0mg/dL – stage 2);
- Grade C – Stage 3 involvement of any organ system (generalized erythroderma; bilirubin 6.1 to 15.0mg/dL; diarrhea > 30ml/kg/day);
- Grade D – Stage 4 involvement of any organ system (generalized erythroderma with bullous formation; bilirubin >15mg/dL; frank blood or melena **or** pain **or** ileus).

TABLE 1: Grading of Acute Graft-Versus-Host Disease

Glucksberg Grade
I – Stage 1 or 2 skin involvement; no liver or gut involvement; Lansky PS 90-100
II – Stage 1 to 3 skin involvement; Stage 1 liver or gut involvement; Lansky PS 70-80
III – Stage 2 or 3 skin, liver, or gut involvement; Lansky PS 50-60
IV – Stage 1 to 4 skin involvement; Stage 2 to 4 liver or gut involvement; Lansky PS 30-40
International Bone Marrow Transplant Registry Severity Index
A – Stage 1 skin involvement; no liver or gut involvement
B – Stage 2 skin involvement; Stage 1 to 2 gut or liver involvement
C – Stage 3 skin, liver, or gut involvement
D – Stage 4 skin, liver, or gut involvement

Legend: PS: performance status. Adapted for children from Rowlings PA, 1997 and Cahn JY, 200511,13

TABLE 2: MAGIC target organ acute GVHD staging in children

Stage	Skin (erythema)	Liver (bilirubin)	Upper GI tract	Lower GI tract (stool output per day)
0	No active rash	<2mg/dL	No or intermittent nauseaa, vomiting, anorexia	< 10ml/kg/day or <4 episodes/dayb
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexiaa	10–19.9ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25 – 50% BSA	3.1-6 mg/dL		20 – 30ml/kg/day or 7–10 episodes/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dL		> 30ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation > 5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

Legend: a. Acute GVHD is suspected if anorexia is associated with weight loss, nausea ≥ 3 days, and/or vomiting ≥ 2 episodes/day for at least 2 days; b. one episode of diarrhea corresponds to approximately 3ml/kg of stool volume in children (< 50 kg). If >50kg, consider an approximate stool volume of 200ml as in adults.

MAGIC: *Mount Sinai Acute GVHD International Consortium*; GI: gastrointestinal tract; BSA: body surface area. Adapted from Harris AC, 2016[16].

PROPHYLAXIS AND BIOMARKERS OF GVHD IN PEDIATRIC PATIENTS

Allo-HSCT practices in children differ from those applied to adults mainly because of the following factors inherent in the pediatric population: lower incidence of acute and chronic GVHD, differences in transplant baseline variables (non-malignant diseases, comorbidities, previous treatments, graft sources) and better thymic function. Since HSCT can treat a wide range of non-malignant diseases in children and GVHD is usually less severe and responds better to treatment in this population, GVHD prophylaxis strategies vary more between pediatric than adult transplant centers, particularly among recipients with malignant diseases[17].

Although several pediatric studies were (or are being) conducted to test new strategies, such as ultralow-dose IL-2[18], sirolimus[19-21], maraviroc[22], individualized mycophenolate mofetil (MMF)[23] and anti-thymocyte globulin (ATG) dosing[24-25], abatacept[26], *ex-vivo* T-cell depletion (CD34 positive selection and/or T-cell subset depletion)[27-29], calcineurin inhibitors (CNI) remain the standard for GVHD prophylaxis in adults and children[30]. Few yet important options have been consolidated in the past decade, the most important of which are: cyclosporine A (CsA) as a single agent for matched sibling donor (MSD) transplants[31] or with rabbit ATG (rATG) for matched unrelated donor (MUD)[32] ones as safe options for children under 12 years old[33]; and post-transplantation cyclophosphamide (PTCy)[34-36] or alpha-beta+ T-cell receptor (TCR) and CD19+ depletion for mismatched related or unrelated donor (haploidentical or MMUD) HSCT[37-38].

Lawitschka and cols.[39] performed a survey capturing different real-life approaches for pediatric GVHD prophylaxis. Single-agent CsA was used for MSD myeloablative HSCT in 47% of the 75 included EBMT centers; most of them used a dose of 1.5 mg/kg twice a day and reported lower CsA blood levels (100-150ng/ml in 37% and 160-200ng/ml in 34%). According to the conditioning regimen, CsA target levels < 200ng/ml were reported for myeloablative conditioning (MAC) by 85% and for RIC by 68% of the responding centers, without a higher target lev-

el during the first weeks. The relapse risk in malignant diseases induced early CsA withdrawal, whereas longer CsA maintenance and higher target levels (> 200 ng/ml) was the policy for non-malignant diseases. Most centers (95%) used CsA with methotrexate (MTX), and 81% used additional ATG for MUD and 96% for mismatched donor (MMD) transplants, while only 21% used this approach for MSD HSCT. Scheduling of MTX and leucovorin rescue varied as follows: 10 mg/m^2 (days +1, +3 and +6) in 37%, 15 mg/m^2 (day +1) + 10 mg/m^2 (days +3, +6 and +11) in 28%, and 25% of the centers used the latter option omitting the day +11 dose. Ex-vivo T-cell depletion was used by 50 centers (positive CD34+ selection in 78% and negative selection in 44%), usually for MMD transplants. Prophylaxis for RIC HSCT, mainly for non-malignant diseases, varied widely; the combination of CsA and MTX was the most frequently used regimen (92%), and 90% used additional ATG. Other agents, such as tacrolimus, MMF and alemtuzumab, were used by 19%, 43% and 23% of the centers, respectively, for aGVHD prophylaxis.

In Brazil, three recent retrospective multicenter studies performed by the *Brazil-Seattle GEDECO Consortium* evaluating outcomes in pediatric HSCT patients observed a low incidence of severe acute and cGVHD. Darrigo Jr and cols.[40] reported an incidence of 11% of grade III-IV aGVHD and of 19% of cGVHD in 37 patients treated with bone marrow transplantation from a MUD for severe aplastic anemia (SAA). GVHD prophylaxis comprised CsA and MTX in 97% plus *in-vivo* T-depletion in 100% of cases. Tavares and cols[41], in turn, showed incidences of grade III-IV aGVHD of 18%, 13% and 17% and of moderate/severe cGVHD of 8%, 22% and 4% after MUD (n = 95), MMUD (n = 47) and umbilical cord blood (UCB) (n= 70) transplants, respectively, in patients undergoing HSCT for acute leukemia and myelodysplastic syndrome. In this study, GVHD prophylaxis consisted of a calcineurin inhibitor (CNI) + MMF or steroids in 90% of UCB transplants and of a CNI + MTX in 80% and 89% of MUD and MMUD transplants, respectively. ATG was used in 57% of UCB, 66% of MUD, and 83% of MMUD recipients. In their haploidentical HSCT study, Fernandes and cols[42] reported incidences of 14% and 16% of grade III-IV acute and chronic GVHD, respectively, in 73 patients with primary immunodeficiency diseases. These patients received PTCy, MMF and a CNI as GVHD prophylaxis, coupled with ATG or alemtuzumab in half of the patients.

As an effective and widely available strategy, PTCy induces functional impairment of alloreactive T-cells supported by highly active suppressive mechanisms, including rapid preferential recovery of regulatory T cells (Tregs), thus preventing donor cells from causing GVHD. Haploidentical HSCT with PTCy has been associated with low rates of GVHD and non-relapse mortality (NRM). Efficacy and overall survival (OS) seem comparable to MUD transplants in a number of published studies, though more robust head-to-head comparisons are still underway. Delayed immune reconstitution after PTCy has been shown to lead to a higher incidence of infectious complications, including CMV infection. Furthermore, decreasing relapse in malignant and graft failure in non-malignant diseases without additional toxicity remains an important challenge. GVHD prophylaxis in this setting consist of PTCY (50 mg/kg on days +3 and +4) plus tacrolimus or CsA (target levels between 5 to 15 ng/mL and 200 to 400 ng/mL, respectively) and MMF (30 to 45 mg/kg divided in 3 daily doses), both from day +5, until 1 year and day+35 post-HSCT, respectively. The addition of rATG (0.5mg/kg on day -9 and 2mg/kg/day on days -8 and -7) may be necessary to overcome engraftment failure in non-malignant diseases, particularly in immunosuppression-naive SAA patients[43].

Prophylactic *in vivo* T-cell depletion with ATG has been associated with decreased GVHD rates in many allo-HSCT settings. Walker and cols.[44] tested the benefit of adding rATG to standard GVHD prophylaxis in a recent randomized, multicenter, phase 3 trial. Included patients (196) had a hematologic malignancy (leukemia, myelodysplastic syndrome, or lymphoma), were between 16 and 70 years of age, and received a MUD or a one-locus mismatched graft at HLA-A, HLA-B, HLA-C, or DRB1 following MAC or RIC. In patients receiving rATG (0.5mg/kg on day -2, and 2mg/kg on days -1 and +1) plus CNI + MTX or MMF, they observed a significant improvement in the incidence of cGVHD (26.3%) as compared to that of the standard GVHD prophylaxis group(41.3%),p=0.032, and in the OS rate (70.6% *vs.* 53.3%); adjusted hazard ratio (HR) 0.56 (95% Confidence Interval - CI: 0.35–0.90, p=0.017) at 24 months. Moreover, cGVHD-free, relapse-free survival (GRFS) at 12 months was 57.6% in the rATG combined group *vs*. 40.2% in the standard GVHD prophylaxis group (p=0.010). Despite decades of clinical study, optimal ATG dosing is yet to be determined. Increasing evidence shows that the current weight-based dosing is suboptimal and that the absolute lymphocyte count (ALC) before the first dose of rATG can determine its clearance and thus drug exposure. Depending on the conditioning regimen (mainly total body irradiation *vs.* busulfan-based conditioning), the ALC before rATG was highly variable. Adult patients with low ALCs

had worse OS compared with those with a higher ALC when receiving rATG. Currently, a historically controlled clinical trial in children (the PARACHUTE study; NTR4960) investigating a fully personalized dosing regimen for rATG is at the analysis stage. The proposed dosing regimen varied from 2 mg/kg to 10 mg/kg, depending on body weight and ALC, starting 9 days before HSCT. A preliminary analysis has indicated an apparent improvement in survival and that early CD4+ T-cell recovery is significantly faster and more robust with individualized dosing[45].

Table 3 summarizes the recommendations for GVHD prophylaxis for MAC, non-myeloablative (NMA), and RIC allo-HSCT in pediatric patients, including peripheral blood stem cell (PBSC) and haploidentical transplants, along with their corresponding levels of evidence and grades of recommendation.

TABLE 3- Recommendations for graft-versus-host disease prophylaxis in pediatric patients

Type of HSCT	Disease/ Graft Source	Prophylaxis Regimen	Level of Evidence
MAC allo-HSCT from HLA-matched related donors	. Malignant - BM	CNI ± short MTX 10mg/m2 (D+1,3,6)*	1b, GR-A
	. Malignant - PB	CNI + short MTX ± rATG	2b, GR- B
	. Non-malignant (BM or PB)	CNI + MTX standard - 15mg /m2 D+1 and 10mg/m2 (D+3,6,11)	1a, GR- A
		(if PB, rATG 2,5 - 5mg/kg can be added)	2b, GR- B
MAC allo-HSCT from HLA-matched or 9/10 unrelated donors	. Malignant - BM	CNI + rATG (4.5mg/kg) ± short MTX*	2b, GR- B
	. Malignant - PB	CNI + rATG (< 6mg/kg) + short MTX	2b, GR- B
	. Non-malignant (BM or PB)	CNI + rATG (< 6mg/kg) +MTX standard	1a, GR- A
		(if UCB: CNI + rATG + MMF)	2b, GR- B
MAC allo-HSCT from related or unrelated donors	. Malignant or non-malignant (BM, avoid PB)	HD PTCy (50mg/kg/day on D+3, D+4) If PB, unrelated, or mismatched donors: add CNI + MMF or MTX	2b, GR -C
RIC or NMA allo-HSCT from related or unrelated donors	. Malignant or non-malignant (BM or PB)	CNI + MTX (as for MAC) or MMF (15mg/kg in 3 daily doses) ± rATG (4-6mg/kg) if PB or unrelated	2b, GR- C
Haploidentical allo-HSCT – Baltimore regimen	. Malignant	HD PTCy (50mg/kg/day on D+3, D+4) plus CNI + MMF	2b, GR- B
	. Non-malignant (avoid PB or mother as donor)	Same as above + rATG (0.5mg/kg on D-9, 2mg/kg/day on D-8, D-7)	3b, GR- C

HSCT: hematopoietic stem cell transplantation; allo-HSCT: allogeneic HSCT; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; NMA: non-myeloablative conditioning; HLA: human leukocyte antigen; BM: bone marrow; PB: peripheral blood; UCB: umbilical cord blood; CNI: calcineurin inhibitor (cyclosporine or tacrolimus); MTX: methotrexate; GR: grade of recommendation; r-ATG: rabbit anti-thymocyte globulin; MMF: mycophenolate mofetil; HD PTCy: high dose post-transplant-cyclophosphamide. *CNI alone or with MTX can be the choice in children < 12 years old after bone marrow transplantation for malignant diseases from HLA-matched donors; δ Since UCB transplantation is rarely used nowadays, the dose and use of rATG should be determined on a case-by-case basis, and mini-MTX can possibly replace MMF.

BIOMARKERS FOR ACUTE AND CHRONIC GVHD

Despite several advances in allo-HSCT over the past few decades, GVHD remains the leading cause of NRM after transplant. Therefore, identifying valid and useful GVHD biomarkers for clinical use is still an unmet need.

GI tract GVHD triggers a systemic inflammatory reaction and is thus the main driver of mortality. Recently, the *Mount Sinai Acute GVHD International Consortium* (MAGIC) validated an algorithm probability (MAP) tool derived from the combination of serum levels of two biomarkers of GI GVHD: ST2 and REG3α. When measured at the time of aGVHD diagnosis, the MAP separates patients into three distinct groups, known as Ann Arbor scores, each carrying a significantly different risk of 6-month NRM. Hence, the MAP can be considered as a "liquid biopsy" of the GI tract damaged by the inflammation caused by GVHD and represents a more accurate quantitation of disease burden than clinical symptoms alone. Moreover, the threshold of probability value ($p \leq 0.291$) calculated from these biomarker blood concentrations, taken 1 week after systemic treatment with steroids, was able to separate patients into groups with low and high probability of 12-month NRM, OS and resistance to steroid treatment at week 4. The MAP can also be calculated at day +7, prior to the onset of aGVHD symptoms in any patient, and can predict NRM better than GVHD-related pre-transplant characteristics, such as HLA mismatch, unrelated donor, recipient age, and intensity of conditioning regimen[46].

Giaccone and cols.[47] summarized the recent evidence on the different types of biomarkers linked to acute and chronic GVHD. The authors highlighted the main markers and their types of interaction, as follows: genetic (minor histocompatibility antigens; association between single-nucleotide polymorphisms and genes involved in innate or adaptive immunity); plasmatic (reduced IL-15; increased: sIL-2R alpha, soluble B-cell activating factor [sBAFF], REG3α, ST2, TNFR1, Elafin, IL-8, CXCL9, CXCL10, CXCL11); cellular (reduced: Tregs, $CD56^{bright}$ Natural Killer [NK] cells, CD27+ memory B cells, follicular helper T cells, invariant NK T cells; increased: CD4/CD8 ratio, Th17 lymphocytes, recent thymic emigrant or RTE CD4+CD45RA+CD31+ T cells, BAFF/B-cell ratio, $CD19+CD21^{low}$ B cells) and others associated with disruption of the microbiota (loss of bacterial diversity; expansion of a single taxon, as that of Enterococci, oral Actinobacteria and oral Firmicutes; and reduced levels of protective intestinal metabolites, such as urinary-3-indoxyl sulfate and butyrate).

Research efforts have been done to better understand the exact mechanism by which ATG prevents cGVHD. In a randomized, multicenter trial conducted by *the Canadian Bone Marrow Transplant Group* (CBMTG), ATG prophylaxis significantly impacted cGVHD cellular markers at day +100 in 40 patients (aged ≥16 years). The ATG-treated group had a significant >10-fold decrease in both naive T helper (Th) cells and RTE Th cells, which has been previously associated with moderate/severe cGVHD, and a 10-fold increase in $CD56^{bright}$ NK_{reg} cells ($p<.0001$). Evaluation of Tregs, conventional Th cells, $CD21^{low}$ B cells, and plasma markers (ST2, OSP, sBAFF, IL2Ra - sCD25, TIM-3, MMP-3, ICAM-1, CXCL10, and soluble aminopeptidase N) revealed no impact of ATG on their concentration at day +100. This analysis suggests that ATG primarily prevents cGVHD through suppression of naive Th cells (CD45RA+ CD4+ T cells), with a concomitant expansion of noncytolytic $CD56^{bright}$ NK_{reg} cells after transplantation[48].

Bronchiolitis obliterans syndrome (BOS) is a pulmonary manifestation of cGVHD associated with high morbidity and mortality due to fibrosis of small airways and respiratory insufficiency. Pulmonary function tests have shown limited value for the diagnosis of BOS, particularly in children, since they are able to identify only the most severe cases. Therefore, plasma proteins correlated with BOS would be extremely valuable to enable early diagnosis, guide treatment choices, and monitor responses. A few cellular and plasmatic markers that correlate with BOS after HSCT, such as lung epithelial proteins, are being proposed for their diagnostic potential: matrix metalloproteinase-3 (MMP-3), Krebs Von Den Lungen-6 (KL-6), BAFF levels, and CD19+CD21low B cells[49].

KL-6 is a glycoprotein expressed on pulmonary epithelial cells that is undetectable in the serum of healthy individuals or only present in very small amounts. However, there is emerging evidence that epithelial cells of the proximal and distal air spaces of sick patients release host defence mediators that can facilitate the initiation of inflammatory airway changes; therefore, KL-6 has been shown to be a useful serum marker for BOS after lung transplantation. Gassas and cols.[50] conducted a prospective study to test KL-6 and other plasma markers in allo-HCT recipients. Thirty-nine pediatric patients (≤ 18 years old) were included. They found that KL-6 serum levels, measured before transplant or at 1 month post-HSCT, were significantly higher in surviving patients who developed BOS *vs.* in those who did not (pre-HSCT: mean, 32.6 U/mL *vs.* 5.8 U/mL, $P < .03$; at 1 month: mean, 52.5 U/mL *vs.* 11.4 U/mL, $p < .04$). KL-6 levels at 3 and 6 months after HSCT remained higher

in the BOS group but were not statistically significant ($p < .12$). The high pre-HSCT levels of KL-6 in patients who later on developed BOS indicate that these patients are predisposed to develop this complication. The authors emphasized the importance of performing serum KL-6 level measurements before transplant and at 1 month post-HSCT with a view to a timely identification of patients at a high-risk for BOS. Such patients may benefit from more frequent pulmonary surveillance and early therapy.

The *Applied Biomarker in Late Effects of Childhood Cancer* study (ABLE/PBMTC 1202)[51] evaluated the immune profiles related to cGVHD and to late aGVHD (L-aGVHD). A peripheral blood immune cell panel and a set of plasma markers analyzed at day +100 correlated well with cGVHD diagnosed according to the NIH consensus criteria (NIH-CC). A total of 241 children were evaluable and categorized as L-aGVHD, cGVHD, active L-aGVHD or cGVHD, and no cGVHD/L-aGVHD. Patients with only distinctive features but defined as having cGVHD by the adjudication committee (non-NIH-CC) had immune profiles similar to those of the NIH-CC. Both cGVHD and L-aGVHD had decreased transitional B cells and increased cytolytic NK cells. Additional abnormalities were observed in cGVHD, such as: increased activated T cells, naive Th and cytotoxic T cells, loss of $CD56^{bright}$ NK_{reg} cells, and increased ST2 and soluble CD13. Active L-aGVHD before day +114 had additional abnormalities in naive Th cells, naive Tregs, and in certain cytokines. On the other hand, active cGVHD had an increase in programmed cell death protein 1 (PD-1)-negative memory Th cells and a decrease in PD-1-positive memory Tregs. An exploratory analysis appeared to show a progression of immune alterations from no cGVHD/L-aGVHD to active L-aGVHD, with the most complex pattern seen in cGVHD. Comprehensive immune profiling might thus allow for the development of more specific strategies to minimize L-aGVHD and cGVHD. The same study group performed an additional analysis to compare T cell populations across age groups and to evaluate the impact of the estimated pubertal status at the time of HSCT. In children, the authors observed a broad suppression of newly formed B (NF-B) cells, whereas adults exhibited an increase in T1-$CD21^{lo}$ B cells and a decrease in T1-$CD24^{hi}CD38^{hi}$ B cells. Pre-pubertal children had elevations of aminopeptidase N (sCD13) and ICAM-1. Treg abnormalities in children appeared to occur primarily in memory Tregs, whereas in adults these abnormalities were seen in naive Tregs. It is probable that abnormalities in sex hormone levels post-transplant have an impact on immune reconstitution, since the onset of puberty seems to be the trigger for the decrease in thymic function. These findings support the role of pre-HSCT age and pubertal stage on the occurrence of cGVHD, and both may explain why pre-pubertal children have lower cGVHD rates, less aggressive disease, and biological differences in the pathways involved in the development of this complication[52]. Table 4 (modified from Cuvelier *et al.*, 2020[52]) summarizes the differences in statistical correlation between cellular and plasmatic biomarkers and cGHVD according to pre-pubertal and pubertal stages at the time of transplant.

TABLE 4 - Cellular and Plasma Markers Significantly Associated with cGVHD According to Pubertal Status

	Pre-pubertal	**Pubertal1**
Naïve T cells		
. Naïve Th cells	Increased	Increased (NS)
. RTE naïve Th cells	Decreased	NS
Newly formed B cells		
. CD21lo B cells	Decreased	NS
. T2 transitional	Decreased	NS
. T3 transitional	Decreased	NS
Peripheral B cells		
. Mature Naïve	Decreased	NS
. Unswitched memory/Marginal-zone like	Increased	Increased (NS)2
. Classical switched memory	NS	Increased (NS)
Regulatory T cells		
. PD1- memory Tregs	Increased	Decreased (NS)
. PD1+ memory Tregs	NS	Increased

. RTE memory Tregs	Decreased	NS
. RTE naïve Tregs	NS	Increased (NS)
Regulatory NK cells	Decreased	Decreased
Cytokines and Chemokines		
. ST23	Increased	Increased
. Aminopeptidase N (sCD13)	Increased	Increased (NS)
. ICAM-14	Increased	NS

[1] Prepubertal was defined as a girl aged <10.9 years or boy <12.4 years and pubertal as a girl ≥ 10.9 years or boy ≥ 12.4 years at the time of HSCT. 2 NS = Not statistically significant due to small number of patients. 3 Supressor of tumorigenicity-2. 4 Intracellular adhesion molecule 1.

FIRST-LINE TREATMENT OF AGVHD

The therapeutic approach toward a patient with aGVHD will depend on the organs and sites involved, GVHD grade, prophylactic regimen used, relative importance of the graft-versus-leukemia (GVL) effect (depending on the baseline disease), as well as on patient-related factors (e.g., renal impairment, coexisting infections, center expertise, and access to therapeutic alternatives)[16].

PEDIATRIC CONSIDERATIONS

Even though the incidence of GVHD in children is generally lower than that in adults, roughly 50% of allogeneic transplants in the pediatric population are for the treatment of non-malignant diseases. In some of these disorders, such as in Fanconi anemia, repair systems are highly dysfunctional, which may impact the occurrence of GVHD. Moreover, specific recommendations both for the diagnosis and treatment of GVHD in children should be taken into account in the approach to these patients, such as the need for: adapting the BSA so as to allow for an accurate assessment of the cutaneous GVHD score; ruling out cases of transient macular rash due to viral infection (most common in children); quantifying diarrhea per episode[53]; and combining, whenever possible and indicated, non-pharmacological therapy (e.g., ultraviolet B phototherapy) and oral, non-absorbable corticosteroids (oral budesonide and beclomethasone), with the aim of reducing both the exposure time to and cumulative dose of systemic corticosteroids[11].

Treatment of grade I aGVHD: the first approach is to optimize the prophylaxis regimen used, by adjusting CNI trough levels and adding topical agents (corticosteroids or tacrolimus) accordingly. Adjuvant supportive therapy with anti-hystaminics for controlling pruritus, for instance, may be helpful. No systemic immunosuppression is recommended[54].

Treatment of grade II-IV aGVHD: the initial treatment does not differ between adults and children. Systemic treatment with methylprednisolone (MP) at a dose of 2mg/kg/day or its prednisone equivalent should be promptly initiated upon diagnosis[55]. Concomitant CNI (CsA or tacrolimus) prophylaxis should not be withdrawn, and trough levels should be checked for. For less severe forms (i.e., grade IIa aGVHD), starting MP at a dose of 0.5-1mg/kg/day is acceptable, with close monitoring and possible escalation up to 2 mg/kg if worsening occurs after 72h[56,57]. Non-absorbable glucocorticoids (beclomethasone and budesonide) have also been used in the treatment of mild upper or lower GI aGVHD (10.0–19.9ml/kg/day or 4–6 episodes/stool output/day in children) as an adjuvant to systemic corticosteroids[58,59] . Unfortunately, only around 60% of patients favorably respond to first-line treatment, and many of such responses are not durable[60]. These patients are considered steroid-refractory and should then undergo second-line therapy.

SECOND-LINE TREATMENT OF GRADE II-IV AGVHD

Second-line treatment is recommended in case of aGVHD progression within the first three days (72h) or of lack of improvement after 5-7 days after initial therapy with MP 2mg/kg/day, in combination with an optimized-level CNI, as mentioned above[30]. Studies on the second-line treatment of aGVHD in children are scarce, predominantly retrospective, with poor historical controls, and, as in adults, highly heterogeneous, with great variability across institutions. Since no superiority of one agent over another has been proven to date in this population, the choice of the most appropriate approach should be individualized and dependent upon the following factors: comorbidities, previous therapy, drug interaction, availability, accessibility, and center expertise[30]. Steroid-refractory aGVHD has typically a poor progno-

sis, both in adults and in children, given the high treatment-failure rates in this scenario. Overall, the average response to second-line agents is around 50%, with a median OS of ~60% at 6-months, with or without active disease[61,62]. The 1-year OS in this population is approximately 20-30%[61]. The main results seen with these agents are depicted below.

MMF: this drug acts by inhibiting the synthesis of guanosine triphosphate, a key enzyme involved in T-cell proliferation. MMF was one of the four drugs tested in the phase II, randomized-controlled *BMT CTN* 0302 trial, while assessing its possible role in first-line therapy in combination with MP.[63] In a subsequent phase III study, *BMT CTN* 0802, no significant benefit was seen in GVHD-free survival, nor in the cumulative incidence of cGVHD at 12 months[61]. Retrospective studies showed complete and partial response (PR) rates of up to 77% at 6-months. MMF may hence be considered in select cases as a second-line approach[64,65].

Extracorporeal photopheresis (ECP): this treatment modality uses ultraviolet A rays to irradiate circulating lymphocytes during leukapheresis after *ex vivo* incubation with 8-methoxypsoralen (8-MOP). This leads to lymphocyte apoptosis (including that of alloreactive T-cells) within 24 hours after reinfusion due to subsequent phagocytosis by antigen-presenting cells (APCs), which produce immunomodulatory effects through cytokine regulation and immune-tolerance induction via Treg expansion, as seen in murine models[66,67]. Of note, there are no solid data pointing to an increase in the risk of opportunistic infections, nor of loss of the GVL effect, with ECP, given its immunomodulatory, as opposed to immunosuppressant, properties[68,69]. Several retrospective studies to date have shown the favorable results of ECP in the management of steroid-refractory aGVHD, with complete response (CR) rates varying between 54 to 75%[67,70]. This is particularly true for cases with skin involvement, in which CR rates reach up to 90%[71]. In a retrospective, multicenter study including 98 patients with steroid-refractory aGVHD receiving either ECP or anti-cytokine therapy, ECP was shown to be superior, with a CR rate of 54% *vs.* 20%, respectively[72]. Another study which included 21 patients undergoing ECP therapy, a CR of 100% and 67%, respectively, was observed for those with grade II/III aGVHD.[73]. In a prospective, phase II study published in 2006, which included 59 patients with steroid-refractory or steroid-dependent aGVHD, a CR was observed in 82% of patients with skin involvement and in 61% of those with hepatic or GI tract involvement[68]. A fairly recent meta-analysis including nine prospective studies and a total of 323 patients showed favorable results, particularly for the treatment of GVHD of the skin (84%) and GI tract (65%)[74]. As for the time to observed response, the Spanish group showed early ECP responses, of which 80% occurred within the first 6 months of therapy. This was further corroborated by Greinix and cols., with a significant response being noted after an average of 4 cycles of ECP[68]. Nonetheless, studies specifically addressing the pediatric population are still lacking. Overall, the current evidence, for both adults and children, support the fact that the clinical response to ECP depends mainly on the grade and extent of aGVHD and on the time until initiation of therapy after diagnosis of refractoriness to first-line steroid therapy[75].

ATG: polyclonal and monoclonal antibody-based therapies are among the most widely used second-line agents for GVHD and with which considerable experience has been gained over the past three decades or so. Nonetheless, response rates seldom reach more than 50%, given that most studies exhibit response rates between 20% and 50%, with slightly better results for cutaneous acute GVHD[76,77].

Anti- IL-2Rα antibody therapy: the potential role of IL-2Rα antibody therapy for aGVHD is based on the molecular structure of this antibody in that its alpha subunit (CD25) is found predominantly in activated (alloreactive) T-cells. Basiliximab, as a chimeric IL-2Rα antagonist, has shown some promising results, with CR rates of up to 71% in a phase I study with a small number of patients[78]. Funke *et al*. observed an overall response rate (ORR) of 80% and a 5-year OS of 30% among 34 patients with refractory grade III-IV aGVHD[79].

TNF antagonists (Infliximab, Etarnecept): TNF antagonists seem particularly useful for the management of steroid-refractory GVHD involving the GI tract, with a number of case series, one of which showed an ORR of 70% in 37 patients[80].

Ruxolitinib: this *Janus kinase* (JAK) inhibitor has been shown fairly recently to be efficacious and safe in the treatment of refractory cases of both acute and chronic GVHD[81,82,83]. It was also shown to exert an inhibitory effect over interferon-gamma (IFN- γ) receptor (IFNGR) signalling pathways, which are known to be implicated in the effect of alloreactive T-cells in the pathogenesis of aGVHD. Similarly, *Janus kinases* (JAKs) are involved in all three pathophysiological phases of aGVHD, since they interfere with common cytokine production and signalling pathways, as well as with the development and function of non-T-cell immune effectors, such as APCs[84]. Importantly, JAK-STAT (*signal transducer and activator of transcrip-*

tion) inhibition in preclinical models showed an improvement in aGVHD, while the GVL effect seemed to remain unaltered, with its obvious advantages[85]. Over the past decade, two pivotal studies – REACH 1 and REACH 2 - enabled ruxolitinib to become, in 2019, the first second-line treatment approved by the *Food and Drug Administration* (FDA) as an alternative to the management of steroid-refractory grade II-IV aGVHD[86,87,88]. *REACH1* was a prospective, phase I, single-arm study which reported an ORR (CR and PR) of 54.9% on D+28 and an OS at 6 months of 73%. Cytopenia and viral reactivation were the most common adverse events observed.[86] *REACH2*, in turn, was a much larger, multicenter, phase III, randomized-controlled study, which compared the efficacy of ruxolitinib (20mg/day) with nine commonly used salvage therapies for steroid-refractory aGVHD (at physicians' discretion). A total of 309 patients were randomized, with a statistically significantly higher ORR at D+28 (62% vs. 39%, OR: 2,64, 95%CI 1.65-4.22, p< 0.0001) and at D+56 (40% vs. 22%; p<0.05) as compared to controls. After a 6-month follow-up period, 10% of patients in the ruxolitinib arm lost their response to therapy, as opposed to 39% in the control group[88]. More recently, ruxolitinib was assessed in a study of 29 pediatric patients with steroid-refractory grade II, III-IV aGVHD or cGVHD and showed rather astonishing results, with response rates of 80%, 82% and 100%, respectively, with initial doses of 5mg or 10mg/day, according to body weight (<15kg or ≥ 15kg), and possible dose escalation to 20mg/day, if tolerable, regardless of weight. Data on the pharmacokinetics of ruxolitinib in this population, however, are still pending in order to better define the optimal dosing of this inhibitor and the most appropriate schedule for immunoglobulin G (IgG) serum level monitoring[89]. Of note, children under ruxolitinib therapy should receive appropriate antimicrobial prophylaxis and be closely monitored and followed up for possible intervening infections.

DIFFERENTIAL DIAGNOSIS BETWEEN ACUTE AND CHRONIC GVHD

The classification of GVHD in classic and late or recurrent forms proposed by the 2005 NIH Consensus[1] was not changed in the 2014 Consensus[3]. It includes: (1) classic GVHD (erythema, maculopapular lesions, nausea, vomiting, anorexia, diarrhea, paralytic ileus, or cholestatic liver disease) that appears before 100 days after HSCT or after donor lymphocyte infusion (DLI), without distinct signs or diagnosed cGVHD; (2) Late, persistent or recurrent GVHD: classical GVHD presentation, which occurs after 100 days of HSCT or DLI (often after decrease or withdrawal of immunosuppression) without distinct signs or diagnosed cGVHD. Overlap GVHD occurs when both acute and chronic GVHD features are present. It is generally correlated with a worse prognosis and an adverse impact on OS. There is no time limit for its onset.

TABLE 5 - Acute and Chronic GVHD Categories

Category		Time of onset	aGVHD	cGVHD
aGVHD	Classic	<100 days	Yes	No
	Persistent/Recurrent/Late Acute	> 100 days	Yes	No
cGVHD	Classic (De Novo/ Quiescent/Progressive)	No limit	No	Yes
	Overlap	No limit	Yes	Yes

Legend: aGVHD: acute graft-versus-host disease; cGVHD: chronic graft-versus-host disease; persistent (previously unresolved aGVHD); recurrent (previously resolved aGVHD); late acute (without prior aGVHD); classic and overlap cGVHD: de Novo (without prior aGVHD); quiescent (previously resolved aGVHD); progressive (previously unresolved aGVHD)

DIAGNOSIS AND INDIVIDUAL ORGAN PRESENTATION OF CGVHD

As a rule, distinguishing between acute and chronic GVHD basically depends on the clinical manifestations rather than the time point at which they present after HSCT[1]. Presenting signs and symptoms may be termed "diagnostic", when they allow for a prompt diagnosis of cGVHD, regardless of any additional testing or organ involvement; "distinctive", which are commonly present in cGVHD and not in aGVHD but are not enough for a definitive diagnosis of cGVHD; and "common", when features of both chronic and acute GVHD are present at the same time[3,90]. A diagnosis of cGVHD is obtained when at least one of such diagnostic manifestations is observed or at least one distinctive manifestation is confirmed with a histopathological examination or with laboratory tests, or, yet, upon specialized evaluation (e.g., with a gynecologist or ophthalmologist) or radiological examination of the same or of different sites[3,90].

From the pathophysiological standpoint, cGVHD involves an array of phenomena comprising inflammation, cellular and humoral responses, and fibrosis. This way, it closely resembles autoimmune diseases of the collagen vascular type. Disease onset is more commonly seen during the first year post-transplant but may also be noted several years after HSCT[9].

Clinical manifestations of cGVHD may be limited to a single organ or site, or may be widespread, with disseminated disease potentially leading to a severe quality of life (QoL) burden for the patient[91,92].

Of note, cGVHD must be clearly differentiated from post-transplant infectious complications, such as those due to fungal or viral infections, or yet from other causes, such as those related to drug toxicity, disease relapse, or secondary malignancy.

It may involve virtually any organ or site, the most common of which being the skin, mouth, hair/scalp, nails, eyes, GI tract, genitalia, liver, lungs, muscles, fasciae, and joints, hematopoietic and immune system, among others[1,3,90]. As mentioned previously, the NIH consensus statements from 2005 and 2014 offer a comprehensive guide for the appropriate identification of the diagnostic, distinctive, and common features of c GVHD, as well as for the grading process based on the specific organ/site involvement observed [1,3].

An accurate diagnosis and grading of cGVHD may be quite challenging, given the uncertainties related to the pathophysiology of this disease and the common coexistence of aGVHD manifestations. This is further aggravated by the lack of a robust validation of the current grading tools and biomarkers for the diagnosis and risk assessment of this post-transplant complication[3].

GLOBAL SEVERITY SCORE OF CGVHD

The NIH global severity score for cGVHD was first proposed in 2005 and later revised and updated in 2014[1,3]. In this grading system, the score varies from 0 to 3 at each organ or site involved, comprising a total of eight sites (skin, eyes, gut, liver, lungs, joints, fasciae, and genitourinary tract) The global score takes into account both the number of organs or sites involved and the severity of involvement at each organ/site[1,3].

According to the total score obtained, the cGVHD observed may be classified as mild, moderate, or severe, which will reflect the degree of impact and functional impairment at each organ or site involved.[3,90]. Importantly, cGVHD should be graded at diagnosis and during follow-up, hence allowing for clinical severity and prognostic re-evaluation in a timely manner[3,90]. Table 6 depicts the global severity scoring system of cGVHD.

TABLE 6: NIH global severity score of cGVHD

Mild cGVHD 1 or 2 organs involved and Individual organ score of no more than 1 and Lung score of 0
Moderate cGV 3 or more organs involved and Individual organ score of no more than 1 OR At least 1 organ (except lung) with a score of 2 OR Lung score of 1
Severe cGVHD At least 1 organ with a score of 3 OR Lung score of 2 or 3
Key points: Skin: the highest of the two scores should be used for calculating global severity. Lung: FEV1 should be used instead of the clinical score for calculating global severity. If the abnormality in an organ is considered to be unequivocally explained by a non-GVHD cause, its corresponding score will be zero and thus not included for calculating global severity. If the abnormality in an organ is attributed to multifactorial causes (GVHD plus other causes), its corresponding score will be used for calculating global severity regardless of the contributing causes (without any downgrading of organ severity score).

Legend: NIH: National Institutes of Health; cGVHD: chronic graft-versus-host disease; FEV1: forced expiratory volume in the first second.
Adapted from: Jagasia MH et al., 20153.

TREATMENT OF CGVHD

No systemic treatment is needed for cases of asymptomatic, mild cGVHD. In such cases, topical steroids, for instance, for skin, mouth, or genital involvement, may be applied, with close monitoring for possible signs of disease progression at other sites so as to avoid clinical deterioration due to suboptimal treatment[3,90]. A prompt intervention might thus help prevent cGVHD progression[3,90].

In patients with three or more organs/sites involved, or with a global NIH score of 2 or more, at whatever site, systemic immunosuppression should be promptly initiated[3,90].

For patients with a diagnosis of *de novo* cGVHD, possible alternatives are to increase the dose of the immunosuppressant being used and/or to add another immunosuppressant[3,90].

To date, chronic GVHD remains one of the main drivers of late post-allogeneic transplant morbidity and mortality. Some of the main risk factors for a higher transplant-related mortality are: multiple organ involvement, low performance status, low platelet count at diagnosis of GVHD (< 100.000/µL), hyperbilirubinemia, cGVHD progressing from prior aGVHD, extensive skin involvement at diagnosis of GVHD, among others[6,90,93,94,95,96].

Patients presenting with cGVHD are more prone to infectious complications due to the intense immunosuppression they are submitted to, as well as the functional asplenia and hypogammaglobulinemia that typically accompany the post-transplant period[90,92]. This results in infections being the predominant cause of mortality in these patients. Therefore, all patients with a diagnosis of cGVHD should receive appropriate *Pneumocystis jirovecii* pneumonia prophylaxis, as well as vaccines against encapsulated bacteria, namely *Meningococcus* sp., *Haemophilus* sp., and *Pneumococcus* sp, coupled with human immunoglobulin replacement at regular monthly intervals, as needed[90,92]. When presenting with fever, patients with cGVHD need to be promptly evaluated and treated, due to the risk of sepsis and of rapid clinical deterioration[90,92].

The main goal of the treatment of cGVHD is to reduce its corresponding symptoms, control disease progression, and prevent harm or disability[3,90]. Treatment intensity will depend on both the extension and severity of the disease. The 2014 NIH Consensus Guidelines addresses the severity criteria and grading of the disease, thus aiding in the decision-making process as to whether topical or systemic treatment should be applied[3]. In patients presenting with only mild symptoms, limited to a single organ or site, it is acceptable to adopt a conservative, watch and wait approach, or to use topical therapy alone, whereas, for patients with a worse clinical picture or multiple organ involvement, systemic treatment is warranted.[3,90]. The management of cGVHD may be quite challenging, and caution should be taken to keep systemic immunosuppression to the least degree possible, with the aim of controlling the disease until immunological tolerance is established between donor and recipient[3]; less immunosuppression allows for a lower rate of severe infections.

Some key points ought to be emphasized when managing cGVHD in the pediatric population, one of which is the potential long-term effects of high-dose steroid therapy. Another aspect is that of children who undergo HSCT for non-malignant diseases, wherein the GVL effect coexisting with GVHD is unnecessary[97,90].

Since cGVHD often involves several organ systems, a multidisciplinary approach to the management of this disease is of at most importance and should generally include physical therapy, psychological, nutritional, dental, social and occupational therapy support[98].

TABLE 7. Indications for systemic therapy of chronic GVHD[3,90,99]

Global severity	High mortality risk *	Systemic therapy
Mild	No	No
Mild	Yes	Yes≠
Moderate	No/Yes	Yes
Severe	No/Yes	Yes

Legend: GVHD: graft-versus-host disease.
* Platelets < 100,000/µL or under steroid therapy at the time of diagnosis of GVHD
≠ A balance between the potential benefit of graft-versus-leukemia effects and the risk of GVHD should be sought

FIRST-LINE TREATMENT

According to the 2014 NIH Consensus criteria, systemic treatment of cGVHD should be administered for cases with: score >2 in any organ, involvement of three or more organs, and mild cGVHD with high-risk features (platelet count <100,000/mm3 and use of immunosuppression at the time of the diagnosis of cGVHD)[94].

First-line systemic treatment consists of 1mg/kg/day prednisone (or its equivalent) and CsA (or tacrolimus), with dose adjustment for serum level[100]. There is no solid evidence that the addition of another immunosuppressant (MMF, azathioprine, or thalidomide) to first-line therapy improves the results, in which case this should not be done[20]. After a two-week period, if there is a response to therapy or the condition is stable, one should start tapering the dose of steroids every other day, with a weekly reduction of 25%, for 6 to 8 weeks, until a dose of 0.1mg/kg/day is reached. According to the *Fred Hutchinson Cancer Research Center* (FHCRC), this dose should be maintained for 2 to 3 months, in case of incomplete response, severe presentation, or GVHD-related risk factors.[99] This should then be followed by a second period of dose tapering, with a dose reduction of 10 to 20% a month, until total withdrawal after 9 to 12 months[99]. When other immunosuppressants are being used concomitantly, these should be sequentially tapered, after steroid withdrawal, for a period of 2 to 4 weeks, until complete withdrawal[90].

Steroid-refractory cGVHD is defined as progression of disease after a two-week period under 1mg/kg/day of steroids, whereas stable disease is considered when a dose of > 0.5mg/kg/day is used for 4 to 8 weeks or when one does not tolerate a prednisone dose < 0.5mg/kg/day[101].

Second-line therapy for cGVHD is indicated when at least one of the following criteria are met: worsening of cGVHD at a primarily involved organ or site, lack of response to therapy after a 1-month period, or inability to reduce the dose of prednisone to levels below 1mg/kg/day for a period of 2 months[97].

SECOND-LINE THERAPY AND NOVEL TREATMENTS FOR CGVHD

There is currently no optimal treatment choice for second-line therapy for cGVHD. Choice of treatment will depend on several factors, such as: organ or site involved, toxicity profile, center expertise, treatment availability, and patient preference. One should not start a third treatment (e.g., immunosuppressant) before an observation period of two to three months so as to better assess response to each therapy[90,99]. The main second-line treatment options for cGVHD are:

- ECP: ECP constitutes an effective treatment modality for refractory or steroid-dependent cGVHD, both in adults andchildren[102]. It is considered a good option for the second-line approach to patients who are dependent upon, intolerant to, or, yet, resistant to corticosteroids. It may also be considered for cases with recurrent infections or at a high risk of relapse of their baseline disease. ECP has been shown to be particularly effective in mucocutaneous cGVHD, with CR rates of up to 80%, as well as a good response in sclerotic forms of this disease[103,73]. Response rates also tend to be high in cGVHD with mouth, eye, and liver involvement, with a response rate of 70%, 60%, and 68%, respectively[104,105]. Moreover, ECP has been shown to enable dose reduction of chronic steroid therapy in select cases[73,106,107,108]. On the other hand, ECP should not be performed in patients with a total white blood cell count of <1000/mm^3, intolerance to 8-MOP, heparin, or citrate, and/or in those who are hemodynamically unstable[109]. ECP has been shown to be well tolerated in children, with a low rate of, typically mild, side-effects, even in low- or underweight patients[110]. Most often, treatment is interrupted due to a lack of an appropriate vascular access, which can usually be managed by insertion of a large-caliber and rigid-type central venous catheter[30]. Hence, ECP is a both feasible and safe treatment option for cGVHD in children, with favorable results. Some studies have suggested the use of ECP as a possible first-line therapy option for refractory or moderate/severe cGVHD in specific clinical situations[111,112].

- Mammalian target of rapamycin (mTOR) receptor inhibitors: the most commonly used agent within this class is sirolimus (rapamycin). It is generally used in combination with a CNI, with response rates varying from 56% to 81% [113]. However, since it is used in association with a CNI, its serum level should be closely monitored, given the increased risk of thrombotic microangiopathy with this combination[113]. Other relatively common side-effects of this medication include dyslipidemia, renal dysfunction, and cytopenia[90]. Caution should be taken regarding potential drug-drug interactions with sirolimus, for which close serum level monitoring and dose-adjustment should be performed accordingly.

- Low-dose MTX: MTX has long been used in a number of autoimmune disorders, with favorable results. This led several investigators to assess its potential role, at low doses, in the management of cGVHD,

both in adults and children[114,115]. Recommended doses vary from 5 to 10mg/m^2 of BSA at weekly intervals or every 3 to 4 days, with partial or complete responses achieved[115,116]. Some authors reported favorable results with a dose of 7.5mg/m^2/week for refractory cGVHD, with a low toxicity profile and allowing for dose tapering of steroids.[114]. These results have also been reproduced in children[115]. MTX was well tolerated and exhibited a low rate of grade III-IV hematologic toxicity and grade II hepatotoxicity[115]. Current studies have shown that the best response rates tend to be obtained in the treatment of skin and mouth cGVHD, with no apparent increase in the risk of relapse of baseline disease[114,115,116].

- Tacrolimus: CNIs are generally used in association with corticosteroids as first-line treatment of cGVHD. Their use as second-line therapy is fairly limited and has provided somewhat modest results[117]. Switching from CsA to tacrolimus has not significantly improved these results, except for a single study which showed a 20% improvement in overall response[118].

- MMF: the ORR in cGVHD with this immunosuppressant has varied between 23 and 79% in several case series[90,119,120,121]. The most often observed side-effects of MMF comprise both hematologic and GI toxicity, including the development of ulcers of the intestinal mucosa[90]. Infection rates also tend to increase with this medication, particularly viral infections[122,65].

- Rituximab: as a chimeric, humanized anti-CD20 monoclonal antibody (MoAb), it exerts its anti-GVHD effect by depleting autoreactive B-cells. A prospective study by Cutler *et al*., 2006, showed favorable response rates of rituximab at a dose of 375mg/m^2 in patients with refractory cGVHD, with the additional benefit of allowing for significant steroid tapering[123]. The best responses were observed for GVHD of the skin, particularly in its lichenoid form, and for musculoskeletal GVHD[123]. Most studies recommend a weekly dose of 375mg/m^2 for 4 to 8 weeks[124,125]. The most common side-effects relate to infusion reactions and infectious complications[123].

- Imatinib: this tyrosine-kinase inhibitor has been used as a potential alternative for the treatment of cGVHD, given its anti-platelet derived growth factor receptor (PDGFR) and anti-transforming growth factor receptor beta (TGFB) effect and, thus, its antifibrotic effect[90]. The current evidence has shown favorable results with the use of imatinib for sclerotic-type cGVHD of the skin[126,127]. The recommended dose varies between 100mg and 400mg/day, which is equivalent to a dose of 65mg/m2 to 260mg/m^2/day in pediatric patients.[90,128] Some of the most common side-effects of this medication include hematologic toxicity, fluid retention, and dyspnea[90].

- Low-dose (100-150cGy) thoracoabdominal irradiation (TAI): given its immunosuppressive and immunomodulatory effects, this therapeutic modality can be used in patients with refractory cGVHD[129,130]. The best responses are seen for mucocutaneous cGVHD, particularly for fasciitis and GVHD of the mouth. TAI has also been shown to allow for systemic steroid tapering[90,130].

- **Ruxolitinib**: this *Janus kinase* (JAK) inhibitor has more recently been shown to be efficacious and safe in the treatment of refractory cases of both acute and chronic GVHD[81,82,83]. In a muticenter study by Gomez *et al*., 2020, ruxolitinib showed an ORR of 57% inr the treatment of cGVHD, but only a 4% CR rate was achieved after a median of 4 weeks follow-up[81]. Steroid tapering was possible among 57% of cases[81]. The OS rate at 1 year was 81%. Of note, this comprised a heavily pre-treated population, with several lines of therapy for GVHD. In a recent study by Yang *et al.,* 2021, which included a total of 53 pediatric patients with acute or chronic GVHD who had had a poor response to prior therapy, ruxolitinib had an ORR of 75.5%, which reached 80.6% in those with cGVHD. Among these, 10 were complete responses and 19 were partial responses[82]. Additionally, a total of 39% of cases were able to have their steroids withdrawn[82]. A possible drawback of ruxolitinib is the potential increase in the rate of opportunistic infections due to its anti-T-cell effect. In a study assessing children with acute or chronic GVHD receiving ruxolitinib, an ORR of 77% and 89% was observed, respectively[83]. In these children, ruxolitinib was shown to increase CD4+-memory B-cells, decrease CD4+-Tregs, decrease CD8+-T-cells, and reduce NK cells, with a resulting increase in the occurrence of infections, with a rate of 54%, 18%, and 13% of viral, bacterial, and fungal infections, respectively[83]. Therefore, children under ruxolitinib therapy for GVHD should receive appropriate antimicrobial prophylaxis and be closely monitored and followed up for possible intervening infections. In a recent publication from Brazil, Ferreira *et al.* reported the experience of ruxolitinib in a cohort of 35 adult patients with corticosteroid-refractory cGVHD from two transplantation centers, with the longest follow-up described to date[131]. The patients had a median of 3 organs affected (range, 1 to 7 organs), with most (64%) having moderate cGVHD. The median number of previous therapy lines was 2 (range, 1 to 6). The ORR was 89% (CR, 26%) after a median of 4 weeks of therapy. The median follow-up was 43 months (range, 11 to 59

months). At follow-up, of the 27 patients still alive, 18 (67%) were free of any immunosuppression, and 6 (22%) were receiving ruxolitinib as their sole immunosuppressive drug. Failure-free survival (FFS) was 77% at 6 months, 68% at 12 months, 54% at 24 months, and 51% at 36 months. Toxicities were mostly hematologic and resolved after dose reduction in most cases, supporting the use of this drug as a safe and effective option for refractory cGVHD[131].

- Ibrutinib: this *Bruton-tyrosine kinase* inhibitor has been extensively studied in the past several years and has been shown to be of benefit in adult patients harboring refractory or steroid-dependent cGVHD. Currently, it is the only *FDA*-approved therapy for adults failing at least one prior line of systemic therapy for cGVHD[132,133,134]. In a study by Waller *et al*., 2019, which evaluated 42 patients with refractory or steroid-dependent cGVHD receiving ibrutinib at a dose of 420mg/day over a follow-up period of 26 months, an ORR of 69% was noted, of which 31% were complete responses and 38% were partial ones[132]. Moreover, a sustained response was observed after 44 weeks of treatment in 55% of these patients. Patients with two or three organs involved had a response rate of 73% and 60%, respectively. Of note, in this study, ibrutinib enabled a dose reduction of steroids to < 0.15mg/kg/day in 64% of patients, and complete withdrawal was possible in 19% of cases[132]. As for the side-effects of ibrutinib, pneumonia, fatigue, diarrhea, nausea and vomiting, muscle cramps, and hematomas were among the most commonly reported ones[132,133]. After a mean follow-up of over 2 years, patients with cGVHD who had failed a prior line of therapy continued to show durable responses while on ibrutinib[132,133]. There are no robust data as yet, however, as to the ideal dose and safety of this medication in the pediatric population. In a retrospective study published in 2020, where 22 children with predominantly moderate or severe cGVHD received ibrutinib at a daily dose of 250mg/m^2 per day, a total of eight (36%) children had their medication withdrawn due to adverse events or died. Among the 14 evaluable patients, 12 (86%) achieved a PR after a follow-up of 6 months. Notably, Epstein-Barr virus (EBV) reactivation occurred in one of these patients and pneumococcal sepsis in another, despite appropriate antimicrobial prophylaxis. The authors concluded that, although the results of ibrutinib for the treatment of cGVHD in children are seemingly promising, further studies addressing the pharmacokinetics of this tyrosine-kinase inhibitor are warranted so as to better define its efficacy and optimal dosing in this population[135].

- Mesenchymal stem cells (MSCs): given their potent immunomodulatory properties, especially for their effector function inhibition, MSCs have risen as a promising alternative for the management of immune-mediated disorders, including GVHD[136]. These cells provide the necessary support for stem cell growth and differentiation within the bone marrow milieu, and they are also able to suppress the proliferation of reactive lymphocytes without Major Histocompatibility Complex (MHC)/HLA restriction[90,137,138]. There have been a number of publications reporting successful responses with MSC infusion for the treatment of cGVHD, with an ORR of around 70% and the additional advantage of enabling dose reduction or complete withdrawal of the prior immunosuppressants being used[139,140]. These results have been particularly encouraging in patients with cutaneous, pulmonary, liver, mouth, and eye involvement[140,141]. Durable response rates have also been reported[142]. In a study by Krasowska-Kwieciena *et al*., 2019, nine children with severe acute or chronic GHVD who were resistant to corticosteroids and second-line immunosuppressants were assessed for their response to MSC infusion[143]. In this study, children received between one and six MSC infusions, with no infusion-related adverse events and an ORR rate of 56% after the first infusion and of 44% after the end of treatment[143] Patients presenting with cGVHD of the skin, GI tract, and liver had a CR rate of 50%, 38%, and 33%, respectively[143].

- Belumosudil: this is a selective oral inhibitor of *Rho-associated coiled-coil kinase-2* (ROCK2), a signaling pathway that modulates inflammatory response by regulating Th17/Treg balance and fibrotic processes, which led to its investigation for the management of cGVHD. Belumosudil reduces type 17 and follicular Th cells via downregulation of STAT3 and enhances Treg function via upregulation of STAT5[144,145]. Jagasia *et al*., 2021, published the results of a phase IIa, open-label, dose-finding study of belumosudil, which enrolled 54 patients with cGVHD who had received one to three prior lines of therapy[144]. The primary endpoint was ORR. The median time from cGVHD diagnosis to enrollment was 20 months. Seventy-eight percent of patients had severe cGVHD, 50% had ≥ 4 organs involved, 73% had cGVHD refractory to their last therapies, and 50% had received ≥ 3 prior lines of therapy. With an overall median follow-up of 29 months, the ORR with belumosudil 200 mg once daily, 200 mg twice daily, and 400 mg once daily was 65%, 69%, and 62%, respectively. Responses were clinically meaningful, with a median duration of response of 35 weeks, and were associated with QoL improvements and corticosteroid dose re-

ductions. Corticosteroid treatment was discontinued in 19% of patients[144]. The FFS rate was 76% and 47% at 6 and 12 months, respectively. The 2-year OS rate was 82%[144]. Belumosudil was well-tolerated, with low rates of cytopenia. There were no unexpected adverse events and no apparent increased risk of infection, including CMV infection and reactivation[144]. Another phase II, randomized, multicenter registration study, published in the same year, evaluated belumosudil 200mg once daily and 200mg twice daily in 66 patients in each group with cGVHD who had received 2 to 5 prior lines of therapy[145]. Overall, median follow-up was 14 months. The best ORR of belumosudil 200mg once daily and 200mg twice daily was 74% and 77%, respectively, with high response rates observed in all subgroups. All affected organs demonstrated complete responses, with a median duration of response of 54 weeks[145]. Adverse events were consistent with those expected in patients with cGVHD receiving corticosteroids and other immunosuppressants[145]. Therefore, selective ROCK2 inhibition with belumosudil was found to be a promising therapy for refractory cGVHD, with a high ORR and OS rate, limited toxicity, and improvement in QoL, by allowing for steroid dose reduction in these patients[144,145]. Belumosudil was thus recently approved by the FDA for the treatment of cGVHD in adult and pediatric patients aged 12 years or older after failure of at least two prior lines of systemic therapy.

OTHER DRUGS

- Baracitinib: this is an inhibitor of *Janus kinase* 1 and 2 (JAK1/JAK2) which was shown to inhibit both the IFNGR and IL-6 receptor (IL6R), resulting in elimination of GVHD in a fully MHC-mismatched allo-HSCT model[146]. Baracitinib can also expand Tregs, by preserving JAK3-STAT5 signaling (thus providing a potential preventive role), and downregulate CXCR3 and Th1 and Th2 cells, while preserving allogeneic APC-stimulated T-cell proliferation[146]. Moreover, baracitinib may also be of benefit in the treatment of established GVHD by promoting intestinal tissue repair via epidermal growth factor receptor (EGFR) effects[147]. Nonetheless, thus far, it has not been approved for the management of GVHD, and further studies are pending.

- Pomalidomide: thalidomide is active in mouse models of cGVHD and has been tested for the prevention and therapy of cGVHD in humans[148]. However, doses expected to be effective were poorly tolerated because of somnolence, neuropathy, and constipation. Pomalidomide is a new immune-modulating drug, with a 4000-fold greater inhibition of TNFα relative to thalidomide, and is well tolerated, without the adverse effects commonly seen with the latter[149]. Several features of pomalidomide suggest it may be useful in treating cGVHD[149]. In a phase II, open label, randomized study, patients with moderate/severe unresponsive or progressive cGVHD exhibited an ORR of 47% at 6 months, with a greater response rate in joint/fascia, followed by skin, GVHD[149]. Further studies may help elucidate its potential role in this setting.

ORGAN-SPECIFIC MANAGEMENT AS AN ADJUVANT THERAPY FOR CGVHD

Specific treatment and supportive care measures directed at individual target organs, such as the skin, genitalia, eyes, and mouth, have been thoroughly addressed in a previous issue of this journal, within the *Consensus Guidelines for hematopoietic stem cell transplantation from the Brazilian Society for Blood and Marrow Transplantation and Cellular Therapy* – SBTMO, which we kindly encourage the reader to access for a deeper look into this matter[150].

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