DIAGNOSIS AND TREATMENT OF GVHD

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INTRODUCTION

Around 50% of patients who undergo a hematopoietic stem cell transplant (HSCT) develop graft-versus-host disease (GVHD), with varying degrees of clinical severity and mortality rates of up to 20%[1,2]. The current guidelines will focus on the diagnosis, staging, grading, prophylaxis, and treatment of acute (aGVHD) and chronic GVHD (cGVHD).

DIAGNOSIS OF ACUTE GRAFT-VERSUS-HOST DISEASE (aGVHD)

The main risk factors for aGHVD are: HLA-mismatch between donor and recipient; gender disparity between donor and patient; conditioning regimen intensity; prophylaxis regimen used; progenitor stem cell source (peripheral blood > bone marrow > umbilical blood cord). The skin, gastrointestinal (GI) tract, and liver are the most commonly affected organs in aGVHD. End-organ manifestations are characterized by a maculopapular rash (skin), nausea, vomiting, anorexia, and diarrhea (gut), and elevated bilirubin, canicular enzyme, and, less often, transaminase levels (liver).

ACUTE GVHD (aGVHD) STAGING AND CLASSIFICATION

The Mount Sinai Acute GVHD International Consortium (MAGIC) has recently allowed for a better standardization of the criteria for classification and data collection related to aGVHD[9]. It is currently regarded as the most appropriate method for the diagnosis, staging, and grading of aGVHD[6,7], as shown in tables 1 and 2, below:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Skin (erythema)</th>
<th>Liver (bilirubin)</th>
<th>Upper GI tract</th>
<th>Lower GI tract (stool output per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No active rash</td>
<td>&lt;2mg/dL</td>
<td>No or intermittent nausea, vomiting, or anorexia</td>
<td>Adult: &lt; 500 ml/day or &lt;3 episodes/day Child: &lt; 10 ml/kg/day or &lt;4 episodes/day</td>
</tr>
<tr>
<td>1</td>
<td>Maculopapular rash &lt;25% BSA</td>
<td>2-3 mg/dL</td>
<td>Persistent nausea, vomiting or anorexia</td>
<td>Adult: 500–999ml/day or 3–4 episodes/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day</td>
</tr>
<tr>
<td>2</td>
<td>Maculopapular rash 25 – 50% BSA</td>
<td>3.1-6 mg/dL</td>
<td></td>
<td>Adult: 1000–1500 ml/day or 5–7 episodes/day Child: 20 – 30 ml/kg/day or 7–10 episodes/day</td>
</tr>
</tbody>
</table>

TABLE 1 - MAGIC Target Organ aGVHD Staging
|   | Maculopapular rash > 50% BSA | 6.1-15 mg/dL | Adult: >1500 ml/day or >7 episodes/day  
Child: > 30 ml/kg/day or >10 episodes/day | 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). |
<table>
<thead>
<tr>
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<th></th>
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<tbody>
<tr>
<td>3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. A diagnosis of aGVHD is suspected when anorexia is associated with weight loss, nausea lasting for at least 3 days, or accompanied by vomiting ≥ 2 episodes/day for at least 2 days; b. one episode of diarrhea corresponds to approximately 200 ml of stool volume in adults and 3 ml/kg in children (< 50 kg).


**Table 2 – MAGIC Overall Clinical Grading of aGVHD**

<table>
<thead>
<tr>
<th>Overall grading</th>
<th>Skin (erythema)</th>
<th>Liver (Bilirubin)</th>
<th>Upper GI tract</th>
<th>Lower GI tract (stool output per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>0-3</td>
<td>2-3</td>
<td>0-1</td>
<td>2-3</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>4</td>
<td>0-1</td>
<td>4</td>
</tr>
</tbody>
</table>

Magic: Mount Sinai Acute GVHD International Consortium  
Adapted from: A.C. Harris et al. / Biol Blood Marrow Transplant 22 (2016) 4e10.

**GVHD PROPHYLAXIS**

Table 3 depicts the main GVHD prophylaxis regimens used in myeloablative, non-myeloablative, and reduced-intensity conditioning allogeneic HSCT, including peripheral blood stem cell (PBSC) and haploidentical transplants, along with their corresponding levels of evidence and grades of recommendation.

**Table 3 - Main GVHD prophylaxis regimens used, with levels of evidence and grades of recommendation**

<table>
<thead>
<tr>
<th>Type of allo-HSCT</th>
<th>Prophylaxis Regimen</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA allo-HSCT from related and unrelated donors</td>
<td>Calcineurin inhibitor and Methotrexate (MTX)9–15</td>
<td>Level 1a, grade of recommendation A</td>
</tr>
<tr>
<td>Calcineurin inhibitor and Mycophenolate Mofetil (MMF)14–19</td>
<td>Level 1a, grade of recommendation B</td>
<td></td>
</tr>
<tr>
<td>High-Dose Post-Transplant Cyclophosphamide (50 mg/kg on D+3 and D+4) 20–24</td>
<td>Level 2b, grade of recommendation C</td>
<td></td>
</tr>
<tr>
<td>RIC and NMA allo-HSCT from related donors</td>
<td>Calcineurin inhibitor and MMF25</td>
<td>Level 4, grade of recommendation C</td>
</tr>
<tr>
<td>HLA-identical allo-HSCT from related and unrelated donors using PBSC as stem cell source</td>
<td>Rabbit Antithymocyte Globulin (rATG) &lt; 6 mg/kg26–33</td>
<td>Level 1a, grade of recommendation A</td>
</tr>
<tr>
<td>Haploidentical allo-HSCT – Baltimore protocol</td>
<td>High-Dose Post-Transplant Cyclophosphamide (50 mg/kg on D+3 and D+4) plus a calcineurin inhibitor and MMF34–36</td>
<td>Level 2b, grade of recommendation B</td>
</tr>
<tr>
<td>Haploidentical allo-HSCT – Beijing protocol</td>
<td>High-Dose rATG (10 mg/kg), MMF, calcineurin inhibitor, and MTX37</td>
<td>Level 2b, grade of recommendation B</td>
</tr>
</tbody>
</table>

allo-HSCT: allogeneic hematopoietic stem cell transplant; MA: myeloablative; NMA: non-myeloablative; RIC: reduced-intensity conditioning; HLA: human leukocyte antigen; PBSC: peripheral blood stem cells.
TREATMENT OF aGVHD

Grade I aGVHD: optimize prophylaxis regimen, adjusting for calcineurin inhibitor trough levels, and add topical agents (corticosteroids or tacrolimus). No systemic immunosuppression is recommended\(^{38}\) – Level of evidence 1b, Grade of recommendation A.

Grade II-IV aGVHD: start systemic treatment with methylprednisolone (MP) at a dose of 2mg/kg/day or its prednisone equivalent\(^{39}\) – Level of evidence 1a, Grade of recommendation A. Concomitant calcineurin inhibitor (cyclosporine or tacrolimus) prophylaxis should not be withdrawn. For less severe forms (grade IIa aGVHD), start MP at a dose of 0.5-1mg/kg/day, escalating up to 2 mg/kg if worsening occurs after 72h\(^{40}\) – Level of evidence 1b, Grade of recommendation A. Non-absorbable glucocorticoids (beclomethasone and budesonide) have also been used in the treatment of mild upper or lower GI aGVHD (500-1000 ml/stool output/day) as an adjuvant to systemic corticosteroids\(^{41,42}\) – Level of evidence 1b, Grade of recommendation A.

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 lack of improvement after 5-7 days after initial therapy with MP 2mg/kg/day\(^{43}\) – Level of evidence 5, Grade of recommendation D. Studies on the second-line treatment of aGVHD are highly heterogeneous, with hardly comparable results, great drug and interrater variability, as well as variability across centers. Since no superiority of one agent over another has been proven to date, the choice of the most appropriate approach should be individualized and dependent upon the following factors: previous therapy, drug interaction, availability, accessibility, and center expertise\(^{44}\) – Level of evidence 2b, Grade of recommendation C. Table 4 shows the main treatment options for the second-line treatment of grade II-IV aGVHD.

### TABLE 4 - Second-line therapy for grade II-IV aGVHD, with levels of evidence and grades of recommendation

<table>
<thead>
<tr>
<th>MMF</th>
<th>Level of evidence 2b, Grade of recommendation C43–46</th>
<th>Complete Response (CR) and Partial Response (PR) rates of up to 77% in 6 months.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracorporeal Photopheresis (ECP)</td>
<td>Level of evidence 2a, Grade of recommendation B47–58</td>
<td>Overall response rates (ORR) of 84% in aGVHD of the skin and 65% in that of the gut</td>
</tr>
<tr>
<td>ATG</td>
<td>Level of evidence 2b, Grade of recommendation C59,60</td>
<td>ORR between 20% and 50%, particularly in aGHVD of the skin</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Level of evidence 2b, Grade of recommendation B61,62</td>
<td>Response rates of approximately 80%, with an overall survival of 30% at 5 years</td>
</tr>
<tr>
<td>Infliximab and Etarnecept</td>
<td>Level of evidence 2b, Grade of recommendation C63</td>
<td>ORR of approximately 70%, particularly in aGVHD of the gut</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>Level of evidence 1b, Grade of recommendation A64–69</td>
<td>REACH2* phase III study showed an ORR of 62% at 28 days, compared to a 39% ORR in the control group</td>
</tr>
</tbody>
</table>

MMF: mycophenolate mofetil; ATG: antithymocyte globulin; GVHD: graft-versus-host disease.
CHRONIC GRAFT–VERSUS–HOST DISEASE (cGVHD)

With a prevalence of 30–70% among allogeneic HSCT recipients, cGVHD remains the main cause of long-term post-transplant morbidity and mortality in this population. The cumulative incidence of cGVHD at 2 years in patients undergoing related or unrelated, bone marrow or peripheral blood stem cell allogeneic HSCT, as defined by the National Institute of Health (NIH) criteria, was 34%^73. 

DIAGNOSIS OF CGVHD AND ITS DIFFERENTIATION FROM aGVHD

The 2014 NIH Consensus recognized two main categories of (acute and chronic) GVHD. The clinical manifestations, and not the actual time of onset of symptoms, are the basis for classifying a case as of acute or chronic GVHD. Table 5 depicts the established categories for acute and chronic GVHD.

<table>
<thead>
<tr>
<th>TABLE 5 - Acute and Chronic GVHD Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>aGVHD</td>
</tr>
<tr>
<td>Classic</td>
</tr>
<tr>
<td>Persistent/Recurrent/Late Acute</td>
</tr>
<tr>
<td>cGVHD</td>
</tr>
<tr>
<td>Classic (De Novo/Quiescent/Progressive)</td>
</tr>
<tr>
<td>Overlap</td>
</tr>
</tbody>
</table>

aGVHD: 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)

CLINICAL SCORING SYSTEM BY TARGET ORGAN

The target organs comprised by the cGVHD scoring system include the skin, mouth, eyes, GI tract, liver, lungs, joints, fasciae, and urogenital (UG) tract. Each organ or body part receives a score within a 4-point (0–3) scale, in which “0” represents absence of involvement and “3” reflects severe involvement74. Table 6 displays each of the cGVHD severity levels.

<table>
<thead>
<tr>
<th>TABLE 6 - Chronic GVHD severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild cGVHD</td>
</tr>
<tr>
<td>Involvement of 1 or 2 organs AND organ score of 1 AND a lung score of 0</td>
</tr>
<tr>
<td>Moderate cGVHD</td>
</tr>
<tr>
<td>≥3 organs with a score of 1 OR at least 1 organ with a score of 2 OR a lung score of 1</td>
</tr>
<tr>
<td>Severe cGVHD</td>
</tr>
<tr>
<td>At least one organ with a score of 3 OR a lung score of 2</td>
</tr>
</tbody>
</table>

cGVHD: chronic graft–versus–host disease.

The use of the 2014 NIH criteria for the diagnosis of cGVHD is both feasible and reliable in pediatric patients. However, specific adjustments in such criteria are needed to better assess the degree of lung and ocular involvement, since pulmonary function tests (PFTs) and Schirmer’s test, respectively, are technically difficult to perform in children younger than 6 years of age.75,76

TREATMENT OF CHRONIC GVHD (cGVHD)

The main criteria for initiating systemic treatment for cGVHD comprise: score >2 in at least one organ, involvement of three or more organs with score 1, lung score 1 or 2, and mild cGVHD with high-risk features (thrombocytopenia <100,000/mm3 and use of immunosuppressants at cGVHD diagnosis). The standard treatment consists of prednisone at a dose of 1mg/kg/day and cyclosporine. Level of evidence 1c, Grade of recommendation A.
**DEFINITION OF REFRACTORINESS TO SYSTEMIC TREATMENT**

Progression of cGVHD after 2 weeks of systemic therapy (prednisone 1 mg/kg/day), stable disease while on prednisone (>0.5 mg/kg/day) for 4-8 weeks, or inability to reduce the dose of prednisone to < 0.5 mg/kg/day. **Level of evidence 5, Grade of recommendation D.**

**INDICATIONS FOR SECOND-LINE THERAPY OF CGVDH**

Worsening of cGVHD manifestations in a primarily involved target organ, absence of any treatment response after one month, or inability to reduce the dose of prednisone to < 1 mg/kg/day within two months. Table 7 depicts the main agents used in the second-line therapy of cGVHD.

<table>
<thead>
<tr>
<th>Extracorporeal Photopheresis (ECP)</th>
<th>Level of evidence 1b, Grade of recommendation A57,81–85</th>
<th>Mucocutaneous manifestations, with complete response (CR) rates of &gt; 80% and significant improvement of sclerotic cGVHD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycophenolate Mofetil</td>
<td>Level of evidence 4, Grade of recommendation B88,87</td>
<td>Overall response rates (ORR) vary between 23% and 79% in several case series</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>Level of evidence 4, Grade of recommendation B88–90</td>
<td>ORR varying between 63% and 81% in several case series</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Level of evidence 2b, Grade of recommendation B80,91,92</td>
<td>Mucocutaneous and musculoskeletal manifestations, with an ORR of approximately 70%</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Level of evidence 2b, Grade of recommendation B92,93</td>
<td>Cutaneous, ocular, and gut manifestations, with an ORR between 50% and 80%</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Level of evidence 4, Grade of recommendation B94,95</td>
<td>ORR varying between 58.8% and 71% in most case series</td>
</tr>
<tr>
<td>Ibrutinib</td>
<td>Level of evidence 2b, Grade of recommendation B96,97</td>
<td>ORR of 67%, with a 21% CR rate</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>Level of evidence 4, Grade of recommendation C98</td>
<td>ORR of 57%, with a 1-year overall survival of 81%</td>
</tr>
</tbody>
</table>

Table 7 - Main agents used in the second-line therapy of cGVHD, with levels of evidence and grades of recommendation.

cGVHD: chronic graft-versus-host disease.

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