# Outcomes of high-grade gastrointestinal graft-versus-host disease posthematopoietic stem cell transplantation in children

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**Abstract**

We explored the clinical course of acute high-grade gastrointestinal graft-versus-host disease in children in a single center. This was a retrospective analysis of 28 pediatric patients who presented with a clinical diagnosis of stage III and IV acute graft-versus-host disease (aGVHD) of the gastrointestinal system (GIS). Generally, skin involvement was the initial manifestation of aGVHD that began in the first 3 weeks of hematopoietic stem cell transplantation (HSCT); on the other hand, GIS involvement predominated after the second week of HSCT. Reported adult data show a survival rate of only 25%; however, our study showed more favorable outcomes in children with a survival rate of 55%. We monitored levels of albumin and immunoglobulin G and observed low levels overall during treatment of unresponsive patients, although only albumin levels were shown to be significantly different. We observed a significant increase in mortality with the use of antithymocyte globulin in GIS aGVHD, although antithymocyte globulin used for graft-versus-host disease prophylaxis had no demonstrable effect on GIS aGVHD mortality. Whether the significantly lower GIS aGVHD mortality among the children recruited in our study than among their historical adult counterparts is a primary result of the specific attributes of the pediatric GIS, or whether it originated from HSCT kinetics remains to be determined by future studies.

**Abbreviations:** aGVHD = acute graft-versus-host disease, GIS = gastrointestinal system, GVHD = graft-versus-host disease, HSCT = hematopoietic stem cell transplantation, SOS = sinusoidal obstructive syndrome.

**Keywords:** children, gastrointestinal graft-versus-host disease, hematopoietic stem cell transplantation

## 1. Introduction

Acute graft-versus-host disease (aGVHD) remains a major complication of hematopoietic stem cell transplantation (HSCT). There is no consensus as to the optimal strategy for managing aGVHD; even though corticosteroids are the recommended initial treatment in the guidelines, a complete response to steroid treatment is seen in about 25% to 70% of cases.[1,2] The high-grade aGVHDs of typically involved organs (skin, gastrointestinal system [GIS], and liver) present specific therapeutic

challenges, and high-grade GIS aGVHD has a distinctive and remarkable place in this context. High-grade GIS involvement usually emerges as insidious diarrhea, and if it is unresponsive to treatment, it gradually worsens into liters of watery diarrhea, which may be complicated by bleeding or sudden ileus. Diagnosis can be challenging because of several other expected causes of diarrhea in the first weeks of HSCT, such as the toxicity of the preparatory regimen, antibiotic-induced diarrhea, and infectious diarrhea caused by *Clostridium difficile* and viruses. The intestinal surface hosts a biological defense system referred to as gut-associated lymphoid tissue, which, unlike the systemic immune system, is composed of Peyer patches, lamina propria, and intraepithelial lymphocytes. Intestinal mucosal injury may trigger an inflammatory response in the intestinal lymphatic system, causing the activation of a cytokine cascade and its progress in a cyclic fashion.[3] Notably, the cytokines released in this condition have the potential to rapidly aggravate symptoms.

The reported incidence of high-grade aGVHD in children varies from 8% to 49%.[4] GIS is generally involved with the skin or can occur alone, and constitutes 35% to 81% of the manifestations of aGVHD.[5,6] The extent of the disease is directly proportional to mortality and only 1 of 4 adult patients with high-grade GIS aGVHD survives the condition.[7,8] Most patients with severe GIS graft-versus-host disease (GVHD) require prolonged hospitalization for supportive care, which gives rise to several social and economic problems. The frequent failure of the modalities used to treat steroid-resistant GIS aGVHD makes preventive measures, including early diagnosis and avoidance of aggravation, the mainstay of the management approach. Few previous studies have aimed to determine the prognosis of this fatal complication,[7,9] particularly in children. Our study sought to analyze the clinical

course of high-grade GIS aGVHD in children. Gastric aGVHD alone has a mild course that is generally responsive to treatment; therefore, we did not include those cases.

## 2. Materials and methods

This is a single-center retrospective review of all pediatric patients undergoing allogeneic HSCT who had a clinical diagnosis of acute GIS GVHD of stage III or higher. Approval for this study was granted by the Ethics Board at the University of Bahçeşehir, Turkey.

The conditioning regimens were mostly based on myeloablative protocols that consisted of busulfan with weight-based, or rarely pharmacokinetic study-based, dosages. Melphalan was used for myeloid malignancies in a dosage of 100 to 140 mg/m$^2$. A reduced-intensity regimen used only for Fanconi anemia consisted of fludarabine with low-dose cyclophosphamide. Almost all patients used cyclosporine A for GVHD prophylaxis in combination with either methotrexate or methylprednisolone. If planned, antithymocyte globulin (ATG) Fresenius (rabbit-based) was used in the pretransplantation period for immunoablation and in vivo T-cell depletion. The haploidentical transplantation conditioning regimen approach did not differ from others; however, when unmanipulated donor stem cells were used, GVHD prophylaxis in 1 patient consisted of post-transplant cyclophosphamide, cyclosporine, and mycophenolate mofetil, while in the other, it consisted of methotrexate, tacrolimus, and methylprednisolone. The patient who was transplanted with CD34-positive selected stem cells did not receive any GVHD prophylaxis.

Our institutional policy for sinusoidal obstructive syndrome (SOS) prophylaxis consisted of continuous heparin infusion, with ursodeoxycholic acid and N-acetylcysteine for 1 month. All patients used acyclovir, ciprofloxacin, and fluconazole prophylaxis for 6 months. Trimethoprim–sulfamethoxazole was used for 1 year. Prophylaxis was extended whenever the immunosuppressant requirement needed to continue because of GVHD development. Neutropenic fever was treated as per the relevant guidelines. Briefly, the initial treatment was monotherapy with piperacillin–tazobactam, and if the fever persisted, it was switched to carbapenems, with caspofungin as a substitute for fluconazole for broad-spectrum antifungal coverage. In case of increased viral load of cytomegalovirus (CMV), preemptive ganciclovir treatment was started for a minimum of 21 days. All patients with severe GIS aGVHD were fed with total parenteral nutrition; oral intake was stopped except for water for at least 3 days initially. It was then supported with oral hydrolyzed formulas in cases without bleeding symptoms. Supportive care for the GIS mucosa consisted of oral zinc, glutamine, and multivitamin suspensions. Intravenous γ-globulin was given whenever the immunoglobulin G level was <400 mg/dL. Albumin infusion threshold was set <2.5 mg/dL; however, this rule was not strict as we administered albumin to patients with levels >2.5 mg/dL and generalized edema.

The diagnosis of aGVHD and the assessment of organ involvement were based on the modified Glucksberg criteria devised at the Keystone Conference.[10] Although the staging of GIS aGVHD for pediatric patients was not discussed at the conference, most pediatric centers have defined it based on volume per kilogram of body weight, as opposed to absolute volume of diarrhea.[11] In the present study, all patients were systematically investigated in order to exclude causes of diarrhea other than GVHD. Stool examination, strip test for rotavirus and

adenovirus, stool culture, polymerase chain reaction (PCR) for CMV in stool, antigen test for amebiasis, and antibody test for norovirus were the initial tests. When no other cause was found, a multiplex PCR stool test was done, which included adenovirus, rotavirus, norovirus, *Giardia*, *Dientamoeba*, *Entamoeba*, *Cryptosporidium*, *Shigella*, *Salmonella*, and *C difficile* species. Initially, in the absence of preceding symptoms of skin aGVHD, the diagnosis of GIS aGVHD was considered suspicious; therefore, we usually scheduled a biopsy of the rectosigmoid colon. However, unlike in adult patients, we were not able to perform biopsy sampling in every pediatric patient due to various physical and social problems, including low platelet counts refractory to transfusions, inability to apply anesthesia due to respiratory problems, and parent reluctance for anal procedures.

For almost all stages of deteriorating GIS aGVHD, our policy was to start methylprednisolone at 2 mg/kg/d and if the condition worsened after 3 days, to switch cyclosporine A to tacrolimus. When there was still no response in 5 to 7 days, either methotrexate 5 to 10 mg/m$^2$/wk for 2 to 3 weeks or ATG Fresenius (rabbit-based) 30 to 50 mg/kg/total for 3 to 5 days was administered. Mycophenolate was added in cases of co-occurring liver or skin involvement; however, if the diarrhea was unresponsive after 7 to 14 days, mycophenolate was stopped due to the possibility of drug-induced diarrhea. As high-grade aGVHD patients are prone to infections because of immunosuppressants and defective barriers, our third-line treatment was generally a combination of photopheresis and mesenchymal stem cells that lack any known marked immunosuppressive effect; any previously started treatment was also simultaneously continued. After observing a superior clinical course using the photopheresis and MSC treatment, we moved this third-line treatment to the second line for the final patients in the study.

Demographics, the regimen used for conditioning and GVHD prophylaxis, clinical characteristics of aGVHD including follow-up, laboratory parameters during aGVHD, treatment modalities used for aGVHD, weekly response assessments, complications of aGVHD, bloodstream infections, and survival data were all recorded. Complete response was defined as the resolution of all symptoms of GIS aGVHD for at least 2 weeks; any other improvement, including to below stage III, was considered a partial response. The cause of death was coded as GVHD if there was no response to treatment or if infection occurred in the context of aggressive immunosuppressive therapy. The definition of fungal infection was based on the European organisation for research and treatment of cancer/mycoses study group criteria.[12] Modified Seattle criteria were used to define veno-occlusive disease.[13] All cases who required preemptive treatment for CMV were recorded as CMV viremia. Hemorrhagic cystitis surveys were performed twice a week and were recorded whenever hematuria of grade 2 or greater was observed for ≥7 days.

### 2.1. Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 16.0.0; SPSS Inc, Chicago, IL). Descriptive statistics of qualitative variables were expressed as frequencies and percentages. The clinical and laboratory data were compared using the Mann–Whitney *U* test. Statistical significance was set at $P < 0.05$.

## 3. Results

Of a total of 331 subjects who underwent an HSCT between December 2010 and August 2015 in Medical Park Antalya Hospital at the Bahçeşehir University School of Medicine, 28

## Table 1

**Patient and transplant characteristics.**

| Characteristic | Value |
| --- | --- |
| Gender | Female, 10 (36%) |
| | Male, 18 (64%) |
| Age, y | Median, 8.6 (1.3–17.6) |
| Diagnosis | Acute leukemia, 7 (25%) |
| | MDS, 7 (25%) |
| | Fanconi anemia, 5 (18%) |
| | Immunodeficiency, 4 (14%) |
| | Hemoglobinopathy, 2 (7%) |
| | Other, 3 (11%) |
| Number of transplants | First transplant, 25 |
| | Second transplant, 3 (1 graft failure, 2 relapses) |
| Donor and HLA match | Sibling, 2 |
| | Parents, 7 (4 full-match, 3 haploidentical) |
| | Unrelated, 19 |
| Gender match | Matched, 12 (42%) |
| | Nonmatched, 16 (58%) |
| HLA match | Full-match, 11 (10/10) |
| | 1 Ag mismatch, 14 |
| | Haploidentical, 3 |
| Cell source | Bone marrow, 19 (68%) |
| | Peripheral blood, 8 (29%) |
| | BM + PB, 1 (3%) |
| | Cord blood, 0 |
| Conditioning regimen | Myeloablative, 23 |
| | Reduced-intensity regimen, 5 |
| Cyclophosphamide dosage (mg/kg) | 20, 1 (for Fanconi anemia) |
| | 40, 4 (for Fanconi anemia) |
| | 120, 9 |
| | 200, 2 |
| Melphalan dosage (mg/m2) | 100–120, 3 |
| | 140, 7 |
| GVHD prophylaxis | CsA and Mtx, 17 (8 alive, 47%) |
| | CsA and Mp, 5 (1 alive, 20%) |
| | Other, 6 |
| Antithymocyte globulin (ATG) | Used, 14 |
| | Not used, 14 |
| ATG dosage, mg/kg | ≤30, 2 |
| | 45–60, 12 |

Ag = antigen, ATG = antithymocyte globulin, BM = bone marrow, CsA = cyclosporin A, GVHD = graft-versus-host disease, GLA = human leukocyte antigen, MDS = myelodysplasic syndrome, Mp = methylprednisolone, Mtx = methotrexate, PB = peripheral blood.

## Table 2

**GVHD characteristics.**

| Characteristic | Value | |
| --- | --- | --- |
| Diagnosis method | GIS biopsy proven, 18 | |
| | Skin biopsy and clinical, 4 | |
| | Only clinical, 6 | |
| Highest stage of GIS aGVHD | Stage 3, 6 | |
| | Stage 4, 22 | |
| Initial involvement | Skin, 15 (54%) | |
| | GIS, 13 (46%) | |
| Skin involvement (highest stage) | Stage 0, 7 | |
| | Stage 1, 2 | |
| | Stage 2, 9 | |
| | Stage 3, 5 | |
| | Stage 4, 5 | |
| Hepatic involvement (highest stage) | Stage 0, 18 | |
| | Stage 1, 0 | |
| | Stage 2, 2 | |
| | Stage 3, 6 | |
| | Stage 4, 2 | |
| Consensus grade | Grade 3, 22 | |
| | Grade 4, 6 | |
| Concomitant organ involvements | Only GIS, 6 | |
| | Only GIS and skin, 12 | |
| | Only GIS and liver, 1 | |
| | GIS, skin, and liver, 9 | |
| Median (range) day of GVHD onset | 14 (5–217) | |
| Median (range) day of GIS GVHD onset | 21 (5–224) | |
| First day of stage 3 GIS GVHD | 32 (7–230) | |
| Immunoglobulin G (mg/dl) (Mean) | | |
| At the first day of GIS GVHD | 725 ± 278 (352–1649) | |
| The lowest level detected | No response, 359 ± 178 (110–726) | $P = 0.8$ |
| | PR + CR, 334 ± 115 (99–566) | |
| The last level detected | No response, 560 ± 203 (164–979) | $P = 0.2$ |
| | PR + CR, 712 ± 297 (395–1455) | |
| Albumin (mg/dl) (Mean) | | |
| At the first day of GIS GVHD | 3.5 ± 0.5 (2.6–4.8) | |
| The lowest level detected | No response, 1.8 ± 0.4 (1.2–2.5) | $P < 0.05$ |
| | PR + CR, 2.2 ± 0.4 (1.7–3.5) | |
| The last level detected | 2.1 ± 0.5 (1.2–3.1) | $P < 0.05$ |
| | PR + CR, 3.5 ± 0.6 (2.6–4.5) | |

CR = complete response, GIS = gastrointestinal system, GVHD = graft-versus-host disease, PR = partial response.

subjects were identified who met the inclusion criteria of severe GIS aGVHD. Patient and transplant characteristics are summarized in Table 1. All patients had a clinical diagnosis of stage III to IV acute GIS GVHD and were mostly diagnosed via rectosigmoid biopsy (75% of patients) (Table 2). In these high-grade GIS aGVHD patients, skin involvement was generally the initial manifestation of the disorder, which began in the first 3 weeks of HSCT, whereas GIS occurred usually after the second week of HSCT. Clinical characteristics of the course of aGVHD are shown in Table 2.

Treatment and outcome characteristics are summarized in Table 3. Complications were frequent, particularly in the unresponsive patients, although the only notable, albeit statistically nonsignificant, difference was seen in the rate of fungal infections, as fungal infections and BK virus–associated hemorrhagic cystitis occurred more frequently in unresponsive patients. Conversely, CMV viremia was more common in treatment-responsive patients, although the difference was also not statistically significant. As for the survival statistics, patients with a history of fungal infections and BK virus–associated hemorrhagic cystitis had a shorter survival (median 4.1 months

[range 1.4–19.9 months]), while patients with CMV viremia had a longer survival (median 7.5 months [range 1.8–56 months[). SOS was more common in unresponsive patients, but the difference did not achieve statistical significance.

Despite the trend for higher IgG levels in patients who were responsive to GVHD treatment, the difference was not significant. However, it should be stated that intravenous immunoglobulin (IVIG) treatment was continuous in patients who were not responsive to the GVHD treatment, and IgG levels were not allowed to decrease to very low levels. In the patients with a complete or partial response, albumin levels were significantly higher than in the unresponsive patients (Table 2).

While only 20% of the patients with involvement of the GIS died, the mortality rate increased relative to the number of involved organs (42% and 66% in 2 and 3 organs involved, respectively). Although the mortality assessments that were made according to the first involved organ revealed a tendency for increased mortality when the first involved organ was the intestine, this tendency did not reach statistical significance.

## Table 3

**Treatment and outcome characteristics.**

| Characteristic | Value | |
|---|---|---|
| The first day of GVHD | Median, 14 d (6–217) in CR+PR | $P=0.5$ |
| | Median, 14 d (5–43) in NR | |
| The first day of GIS GVHD | Median, 24 d (6–224) in CR+PR | $P=0.2$ |
| | Median, 19 d (5–43) in NR | |
| The first day of stage 3 GIS GVHD | Median, 29 d (7–230) in CR+PR | $P=0.5$ |
| | Median, 35 d (15–65) in NR | |
| Actual treatment before the initial GIS symptom | No treatment, 2 | |
| | CsA, 6 | |
| | CsA+Mp, 12 | |
| | MMF+Mp, 4 | |
| | Other, 4 | |

| | | Used | | Not used | |
|---|---|---|---|---|---|
| **Treatment modalities** | | **Dead** | **Alive** | **Dead** | **Alive** |
| Photopheresis | 20 | 8 | 12 | 5 | 3 |
| Mesenchymal stem cells | 18 | 8 | 10 | 5 | 5 |
| ATG | 6 | 6 | 0 | 7 | 15 |
| Mtx | 6 | 3 | 3 | 10 | 12 |
| Sirolimus | 6 | 2 | 4 | 11 | 11 |
| Other (rituximab, infliximab, etc.) | 3 | | | | |

| Complications | NR | CR+PR | P |
|---|---|---|---|
| Fungal infections | 7 (53%) | 2 (13%) | 0.07 |
| VOD | 4 (30%) | 0 (0%) | 0.17 |
| CMV viremia | 7 (53%) | 11 (73%) | 0.38 |
| Hemorrhagic cystitis (BK virus) | 8 (61%) | 6 (40%) | 0.33 |
| Bloodstream infections | | | |
| MR-CNS | 6 | | |
| *Acinetobacter* | 3 | | |
| *Candida* | 4 | | |
| *Enterococcus* | 3 | | |
| *Pseudomonas* | 2 | | |
| Other | 7 | | |
| Overall response | | | |
| NR | 13 (46%) | | |
| PR | 4 (14%) | | |
| CR | 11 (40%) | | |
| Survival | OAS | | |
| Dead | 13 (46%), 2.4 mo (1.4–6.4) | | |
| Alive | 15 (54%), 17.3 mo (4.0–57.0) | | |
| Cause of death | | | |
| GVHD | 11 | | |
| Other | 2 (VOD, candidal sepsis) | | |

ATG = antithymocyte globulin, CMV = cytomegalovirus, CR = complete response, CsA = cyclosporin A, GIS = gastrointestinal system, GVHD = graft-versus-host disease, MMF = mycophenolate mofetil, Mp = methylprednisolone, Mtx = methotrexate, MR-CNS = methicillin-resistant coagulase-negative staphylococci, NR = nonresponse, OAS = overall survival, PR = partial response, VOD = veno-occlusive disease.

Similarly, the mortality rate was higher, albeit statistically nonsignificant, in patients with GIS involvement within 3 days of skin involvement, compared to in those with GIS involvement beyond 3 days (data not shown).

When the applied treatments were immunosuppressive-based, the mortality rate increased further, most significantly with the use of ATG ($P=0.01$) (Table 3). Despite a low overall sample volume that precludes solid conclusions, it was observed that mortality was not increased with sirolimus use, and that mortality actually tended to decrease with the use of immunomodulatory methods such as photopheresis and mesenchymal stem cell use.

## 4. Discussion

In children, gastrointestinal problems require a more complex management approach since their GIS is still in development, even in adolescence. In fact, their systems differ considerably from those of adults, as they show slower gastric passage, a larger mucosal surface area, and lower levels of pancreatic enzymes.[14] Although the extent to which these dissimilarities affect prognosis in high-grade GIS aGVHD is currently unclear, Castilla-Llorente et al[7] reported survival rate of only 25% in adults, while children showed more favorable outcomes with a survival rate of 55%. Our study concurs with their results. In contrast to the classical teaching that mucosal injury secondary to high-dose preparatory regimens is directly proportional to GVHD severity,[15] Castilla-Llorente et al used reduced-intensity conditioning in almost half of their study population. Our patients largely underwent myeloablative therapy, and the majority of our study population consisted of unrelated transplantations and haploidentical transplantations. Moreover, most of our patients had been transplanted from sex-mismatched donors. Although the

better prognosis in children with high-grade aGVHD than in adults in spite of such unfavorable characteristics[16,17] suggests that other factors related to fundamental GIS differences that are independent of graft-versus-host pathogenesis in children may play a role, the frequent use of bone marrow as a stem cell source may also have been influential. Castilla-Llorente et al used peripheral stem cells in 83% of patients with high-grade GIS aGVHD, whereas we used them in 32% of such patients.

In the present study, we explored the clinical course of high-grade GIS aGVHD in children in a single center. Similarly to previous reports, while GIS was the target organ most related to increased mortality and morbidity, skin was usually the first organ of involvement. In general, aGVHD began at a median of 14 days after transplantation, and signs and symptoms of GIS involvement were added to the clinical picture 1 week later, which became worse 10 days after their onset. Although patients with GIS involvement that began within 3 days of the skin involvement appeared to have increased mortality, no statistically significant difference was found between the mortality rates of the patients with GIS signs and symptoms that occurred within the first 3 days and mortality rates of those in which they occurred later. The lack of any significant mortality impact of the timing of GVHD onset and the timing of the addition of GIS aGVHD to the clinical picture in both treatment-responsive and unresponsive patients suggests that GIS aGVHD follow-up and preventive measures should be continued not only in the short term but also in the long term.

Although the literature data suggest that the use of melphalan in particular may result in severe aGVHD,[18,19] we did not observe this impact of the use of melphalan, cyclophosphamide, or ATG as part of the preparatory regimen. However, it is still possible to conclude that mortality tends to increase in patients using cyclophosphamide, while it tends to decrease in those using ATG as part of the preparatory regimen. Despite the fact that the use of ATG in the preparatory regimen typically reduces the rate of chronic GVHD,[20] more patients are needed to show this favorable effect of ATG on high-grade GIS aGVHD. Since all patients receiving low-dose cyclophosphamide had Fanconi anemia, we consider it inappropriate to compare low-dose and high-dose cyclophosphamide.

Protein-losing enteropathies are characterized by the loss of certain proteins such as albumin and γ-globulins usually secondary to erosive disorders of the intestinal surface.[21] As intestinal aGVHD is a typical form of protein-losing enteropathy, we monitored albumin and immunoglobulin G levels. While both proteins showed low levels in treatment-unresponsive patients, only albumin levels showed a significant difference. The association between protein loss and mortality is likely due to greater intestinal mucosal injury and severity of the disease in unresponsive patients. One should bear in mind that protein loss may be rapid and severe in patients with intestinal aGVHD, and therefore albumin and IVIG support should be provided to avoid complications. As the rapidity of protein loss will become apparent during patient follow-up, levels should be monitored carefully, with measurements taken as often as daily if needed.

High-grade GIS aGVHD has a high mortality rate, and although many novel treatment methods have been tried, no marked improvement has been achieved with respect to mortality.[7,22] In spite of the fact that these rather immunosuppressive-based methods may achieve clinical improvement, they cannot provide much survival benefit due to increased rate of infection. Thus, we recently opted not to administer any additional immunosuppressive therapy beyond tacrolimus as a calcineurin inhibitor and methylprednisolone 2 mg/kg/d that is rapidly added to the regimen. In the case of treatment unresponsiveness within 3 to 5 days of the last added drug, we proceeded with photopheresis followed by mesenchymal stem cell administration. Overall, we gave preference to therapies with immunomodulatory rather than immunosuppressive properties. While the difference was statistically nonsignificant, both treatment modalities were associated with lower mortality, as reported in the literature.[23,24] Van Lint et al [25] showed that increased mortality was associated with the use ATG in intestinal aGVHD. We also observed a significant increase in mortality with the use of ATG in GIS aGVHD, although when ATG was used for GVHD prophylaxis, no demonstrable effect on GIS aGVHD mortality was observed. Studies have shown that intense immunosuppressive therapies do not confer much survival benefit despite a decrease in GVHD activity.[26–29] In our study, ATG was shown to increase mortality, but the use of methotrexate for aGVHD treatment did not affect survival. Sirolimus is an immunosuppressive agent that is increasingly used owing to its ability to increase Treg cells.[30,31] Although sirolimus was shown to prolong survival in our study, our sample size was small, and further studies with a larger sample size should be performed.

High-grade GIS aGVHD is characterized by frequent complications that arise from the nature of the disorder itself as well as the applied treatments. Disrupted intestinal mucosal integrity and simultaneous immunosuppressive therapy increase the incidence of infections, and disturbed intestinal flora leads to vicious cycles of bacterial overgrowth. As expected, treatment-unresponsive patients experienced more complications than did responsive patients, although the difference in the rate of complications was not statistically significant. Often, the hepatic dysfunction in HSCT patients is multifactorial and does not meet the classic criteria for GVHD or SOS. In the present study, the time of the initial symptoms, refractory thrombocytopenia, and concurrent fluid overload led us to diagnose the hepatic dysfunction as SOS rather than GVHD. The increased incidence of SOS in our unresponsive patients may be considered a reflection of the intestinal and hepatic injury induced by the preparatory regimen. As expected, unresponsive patients had a greater, albeit statistically nonsignificant, incidence of fungal infections and hemorrhagic cystitis caused by the BK virus, due to more intensive immunosuppressive therapy. While this creates a necessity for frequent viral and fungal screening tests in high-grade GIS aGVHD, especially when a steroid is being administered, further studies should be conducted to determine the role of prophylactic approaches. CMV viremia was more common in responsive patients, although the difference did not reach statistical significance. These findings were consistent with the established early course of the disease in children when fungal infections and BK virus–associated hemorrhagic cystitis are more common, and the late stage when CMV viremia is more common. The disease also appears to cause a greater incidence of CMV viremia in responsive patients who survive for a longer period.[32,33]

Intestinal inflammation secondary to GVHD creates marked alterations in intestinal flora. A reduced diversity of flora is associated with increased GVHD-associated mortality.[34] While the antibiotics frequently used for GVHD may contribute to reduced flora diversity, mucosal injury associated with aGVHD allows for bacterial translocation into the bloodstream. Additionally, immunosuppressives increase the incidence of infections. A thorough understanding of the relative increase in the

frequency of certain types of infections may inform clinicians as to prophylactic antibiotic use. As expected, the majority of infectious agents in our study population were members of the intestinal flora that became pathogenic as a result of the disrupted gastrointestinal mucosal integrity. Methicillin-resistant coagulase-negative *Staphylococcus*, the second most common infectious agent, was also expected to affect our study population owing to the frequent use of catheters and interventions, as well as an increased number of risk factors including mucosal injury and malnutrition.[35,36] Interestingly, despite the use of fluconazole, an agent commonly used for antifungal prophylaxis, *Candida* was also among the common pathogens. These factors must be considered in terms of the development of infections in patients with GIS aGVHD; broad-spectrum antibiotics with anaerobic action should be selected in patients with fever; glycopeptide need should be evaluated in cases of unresponsive fever, and studies examining the efficacy of prophylactic caspofungin use in patients with GIS aGVHD should be conducted with the consideration that fluconazole prophylaxis may fail to prevent *Candida* infections and that the guidelines recommend using caspofungin for *Candida* infections.[37]

There are some limitations to our study. First, our study population consisted of a largely heterogeneous patient population with significant variations with respect to disease properties, donor sex, preparatory regimen, stem cell source, and HLA compatibility. Treatment response may have been modified by these factors. Second, the applied treatment was a highly heterogeneous multidrug regimen and the study sample was small, making it impossible to conclude which treatment regimen was beneficial and for which patient group. Third, although this disease is frequently reported, it still lacks a specific diagnostic method.[38] While the most common organisms causing diarrhea were excluded and the majority of patients underwent biopsy in our study, it should nevertheless be noted that some patients might have suffered from prolonged diarrhea secondary to non-GVHD factors.

Although GIS aGVHD is associated with increased mortality, no significant advancements have been made in terms of optimizing its management for many years. It is obvious that the intestinal system requires a different management approach than other systems in the context of this disorder, since it contains bacteria and cytokines that lead to the initiation and perpetuation of aGVHD. Every episode of diarrhea starting 1 week after transplantation should be considered a manifestation of GIS aGVHD, and further tests should be done immediately, including a biopsy whenever needed. Whether the significantly lower GIS aGVHD mortality among the children recruited in our study than among their historical adult counterparts is a primary result of the specific attributes of the pediatric GIS, or whether it originated from HSCT kinetics remains to be determined by future studies with larger, more heterogeneous study samples.

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