Diagnosis of Intestinal Graft-versus-Host Disease and Thrombotic Microangiopathy after Allogeneic Stem Cell Transplantation

Minami Yamada-Fujiwara,1,4 Koichi Miyamura,1,6 Tohru Fujiwara,2 Yasuo Tohmiya,5 Katsuya Endo,3 Yasushi Onishi,1 Kenichi Ishizawa,2 Junichi Kameoka,1 Masafumi Ito7 and Hideo Harigae1

1Department of Hematology and Rheumatology, Tohoku University Graduate School of Medicine, Sendai, Japan
2Department of Molecular Hematology and Oncology, Tohoku University Graduate School of Medicine, Sendai, Japan
3Department of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan
4Department of Hematology, Sendai Medical Center, Sendai, Japan
5Department of Hematology, Miyagi Cancer Center, Sendai, Japan
6Department of Hematology, Japanese Red Cross First Hospital, Nagoya, Japan
7Department of Pathology, Japanese Red Cross First Hospital, Nagoya, Japan

Severe diarrhea is a serious complication after allogeneic hematopoietic stem cell transplantation (HSCT). Acute graft-versus-host disease (GVHD) has been one of the major causes of diarrhea after HSCT, which is triggered by donor-derived cytotoxic T-lymphocytes. On the other hand, intestinal thrombotic microangiopathy (TMA) sometimes coexists with acute GVHD, and intensified immunosuppression to treat acute GVHD could exacerbate intestinal TMA, presumably through the vascular endothelial cell damage. The differential diagnosis between intestinal TMA and acute GVHD of the gut has mainly relied on the pathological findings, as clinical diagnosis of intestinal TMA has not been established. Therefore, we aimed to assess the feasibility of our clinical diagnosis for the patients with diarrhea after HSCT. We made tentative clinical criteria for intestinal TMA and acute GVHD of the gut, based on the clinical manifestations, laboratory data and colonoscopic findings, and started treatment before pathological diagnosis were made. Subsequently, a pathologist retrospectively assessed the accuracy of clinical diagnosis in a blind manner. In this study, we enrolled 19 patients complicating watery diarrhea after HSCT, and diagnosed as having acute GVHD (n = 10), intestinal TMA (n = 3), or both (n = 6) according to our criteria. We demonstrated that our clinical diagnosis for intestinal TMA and acute GVHD of the gut was overall correct, in terms of the response to the therapy and the pathological diagnosis. The present study may provide a clue on making clinical diagnosis of patients with watery diarrhea after HSCT, which enables us to start a prompt therapy.

Keywords: acute graft-versus-host disease; colonoscopy; hematopoietic stem cell transplantation; intestinal thrombotic microangiopathy; pathological diagnosis

by tacrolimus without irradiation. Therefore, it seems possible to consider that the gut could be a target for TMA, and the use of calcineurin inhibitors such as cyclosporin A and tacrolimus could exacerbate its symptom. As the reduction of immunosuppressants is an opposite treatment for acute GVHD, differentiating intestinal TMA from acute GVHD is important.

Pathological examination of the intestine seems essential to make a diagnosis of intestinal TMA, but it usually takes a time for the analysis. Therefore, in our institute, if patients developed diarrhea more than 0.5 L/day, we made a clinical diagnosis based on clinical manifestations, laboratory data and colonoscopic findings and started treatment. However, it remains unproved whether clinical diagnosis of intestinal TMA and acute GVHD of gut predicts the pathological diagnosis. Therefore, we designed a study to determine the accuracy of our clinical diagnosis of patients who showed watery diarrhea after HSCT by retrospectively reviewing the correlation between their clinical diagnosis and pathological findings as well as clinical outcome.

**Patients and Methods**

**Patients and Study Protocol**

Between March 2001 and March 2006, 73 patients underwent HSCT at the Department of Hematology and Rheumatology in Tohoku University Hospital (Fig. 1). Among them, 20 patients (27.4%) showed watery diarrhea more than 0.5 L/day after HSCT and all received colon fiber examination. Based on colonoscopic findings and laboratory data, patients were judged as having GVHD, TMA or both. A patient, whose diarrhea was considered mainly due to cytomegalovirus enterocolitis, was not included for the analysis. In the patients with GVHD, the addition of prednisolone (PSL) was principally considered, but not indispensable, depending on patients’ general status. On the other hand, a calcineurin inhibitor was reduced in cases with intestinal TMA. In the mixed type, the reduction of calcineurin inhibitors and the addition of steroid or mycophenolate mofetil (MMF) were adopted.

On January 2006, all biopsy specimens of the gut were retrospectively examined by a pathologist (Dr. Masafumi Ito), without any clinical information. Of 20 diarrhea patients, one patient was excluded for the analysis because the biopsy specimen was not suitable for pathological diagnosis. Thus, the 19 patients’ records as well as pathological results were retrospectively analyzed in the present study. Characteristics of 19 patients are shown in Table 1. All patients gave written informed consents.

**Colonoscopic examination**

A colonoscopy, including mucosal biopsies, was conducted for all patients as described previously (Oomori et al. 2005).

![Diagram](https://via.placeholder.com/150)

**Fig. 1. Study design.**

Patients complicating watery diarrhea (0.5 L/day or more) after HSCT were diagnosed as having TMA, GVHD or mixed type based on physical examination, laboratory and colonoscopic findings. In the patients with intestinal TMA, calcineurin inhibitor was reduced, while the addition of prednisolone was principally considered for the treatment of GVHD. In the mixed type, the reduction of calcineurin inhibitors and the addition of steroid or mycophenolate mofetil (MMF) were adopted. On the other hand, biopsy specimens of the gut were retrospectively analyzed by the pathologist in a blinded manner, and then a correlation of clinical diagnosis/outcome and pathological results were analyzed.
Clinicopathological Analysis of Intestinal TMA and GVHD

As soon as the occurrence of diarrhea, the diagnosis of intestinal TMA and/or GVHD was made comprehensively, according to physical examination, laboratory data and colonoscopic findings. Severe abdominal pain requiring morphine, bloody stool, and an occurrence of diarrhea during steroid therapy were possible signs of intestinal TMA. Laboratory findings for the diagnosis of TMA were basically based on the international criteria for TMA (Ho et al. 2005; Ruutu et al. 2007). However, few patients fulfilled the criteria, presumably because they were applied for the diagnosis of completed systemic TMA. Thus, we defined it as “possible TMA” if any two or more of the criteria, including increased LDH, thrombocytopenia, reticulocytosis and anemia without bleeding, RBC fragmentation > 1.2%, were observed (Table 2). Endoscopic findings suggestive of intestinal TMA include i) mucosal petechiae or ii) diffuse exfoliation, which would reflect ischemic colitis (Scowcroft et al. 1981) (Fig. 2A-c,e,f), whereas those of GVHD include i) mucosal edema, ii) loss of transparency of vascular pattern, and iii) mucosal defects visualized by indigo carmine stain (Cruz-Correa et al. 2002; Oomori et al. 2005) (Fig. 2A-a,b). Both findings were observed in the mixed type (Fig. 2A-c,d). Diagnosis of clinical intestinal TMA was made from colonoscopic findings and at least one positive finding of physical examination or laboratory “possible TMA”. Diagnosis of clinical intestinal GVHD was made primarily from colonoscopic findings. Accordingly, patients with diarrhea was diagnosed and treated as described above. Colonoscopy and biopsy of colon were performed in all patients, however, none of these cases required changes of the treatment owing to the pathological results.

**Response criteria**

Evaluation of the response to the therapy was made primarily two weeks after the initiation. However, the response to the second step (ex. addition of MMF) was assessed from two weeks to four weeks further. Decrease of the diarrhea below 0.5 L/day was qualified as positive response.

**Histopathological examination**

Histopathological evaluation for 19 patients with diarrhea was performed on formalin-fixed paraffin-embedded sections. Hematoxylin-eosin stained sections were reviewed retrospectively by the pathologist without clinical information. Microangiopathy with ischemic (non-inflammatory) crypt loss was diagnostic of intestinal TMA (Inamoto et al. 2009). Ischemic changes caused by microangiopathy were as follows: non-inflammatory crypt degeneration with detachment and apoptosis of epithelial cells, wedge-shaped segmental injury, and interstitial hemorrhage or fragmented red blood cells (Fig. 2B-a, b). Pathological diagnosis of GVHD was made by the presence of apoptosis with intraepithelial lymphocytosis (Fig. 2B-c).

**Results and Discussion**

Ten patients were diagnosed as acute GVHD on the basis of clinical and endoscopic findings. None of the patients were pretreated by PSL. Among them, 9 patients were treated with steroid administration and 8 patients responded. The non-responder and the other one patient were improved gradually by the supportive care. Total response rate in this group was 100%. Survival rate at 1 year after HSCT is 90%. The pathological findings of GVHD were recognized in 6 patients (60%). Pathological findings, which suggest ischemic changes, were also pointed out in 9 patients (90%), although 7 were confined to subtle findings. These results suggested that clinical diagnosis of GVHD seemed generally correct, although the pathologist found some ischemic changes in almost all patients. The coexistence of subtle ischemic changes among GVHD cases might result from the use of conditioning regimen and calcineurin inhibitors, though the presence of GVHD itself could not be excluded (Biedermann 2008; Tichelli and Gratwohl 2008).

Among 6 patients who were clinically diagnosed as mixed type, 4 patients (patients 12, 13, 14, and 15) were improved by the reduction of calcineurin inhibitors and the addition of steroid or MMF (response rate: 66.7%).

### Table 1. Characteristics of patients with diarrhea after HSCT.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>9/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Median 35, Range 15-57</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Acute myelogenous leukemia 10, Acute lymphoblastic leukemia 5, Myelodysplastic syndrome 1, Non-Hodgkin’s lymphoma 1, Chronic myelogenous leukemia 1, Renal cell carcinoma 1</td>
</tr>
<tr>
<td>Stem cell source</td>
<td>Related/Unrelated 9/10, HLA identical/mismatched donor 10/9, BM/PB/CB 9/7/3</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td>Myeloablative/Nonmyeloablativeb 14/5</td>
</tr>
<tr>
<td>GVHD prophylaxis</td>
<td>FK506 + MTX 15, FK506 + MTX + PSL 1, CyA + MTX 2, CyA 1</td>
</tr>
</tbody>
</table>

BM, bone marrow; PB, peripheral blood; CB, cord blood; GVHD, graft-versus-host disease; MTX, methotrexate; PSL, prednisone; CyA, cyclosporine A.

bMyeloablative: CY (cyclophosphamide) (120 mg/kg) + TBI (total body irradiation) (12 Gy) 11; Ara-C (cytosine arabinoside) (8 g/m²) + CY (120 mg/kg) + TBI (12 Gy) 2; BU (busulfan) (8 mg/kg) + CY (120 mg/kg) + TBI (10 Gy) 1.

Nonmyeloablative: Flu (Fludarabine) (125 mg/m²) + L-PAM (melphalan) (180 mg/m²) 1; Flu (125 mg/m²) + L-PAM (140 mg/m²) 1; Flu (125 mg/m²) + L-PAM (80 mg/m²) + TBI (4 Gy) 1; FLAG + Ida (Idarubicin) 1. Flu (150 mg/m²) + CY (120 mg/kg) + TBI (2 Gy) 1.

### Diagnostic criteria of intestinal TMA and GVHD

As soon as the occurrence of diarrhea, the diagnosis of intestinal TMA and GVHD prophylaxis was 33.
Pretreatment with PSL for other cause than GVHD was administered in patients 11, 12, and 16. The dose of PSL administration was 1 mg/kg for patient 13, and 2 mg/kg for other patients. MMF was administered in 2 patients (patients 15 and 16) at the dose of 1,000 mg/day. Concurrently with the clinical results, these mixed-type cases were well correlated with pathological findings. Comparing to the GVHD cases, the extent of ischemic changes were more obvious pathologically in mixed-type cases. Therefore, for the mixed-type patients, the reduction of calcineurin inhibitor and/or the addition of MMF, which has less toxicity to vascular endothelial cells (Eugui and Allison 1993), might improve the prognosis. Further prospective studies are warranted to address these points.

The clinical diagnosis of intestinal TMA was made in 3 patients (patients 17, 18, and 19) and was in complete accord with the pathological diagnosis (Table 2). Two patients were pretreated with PSL at the onset of diarrhea (patients 17 and 18). Among them, one patient (patient 17) improved after the reduction of tacrolimus (response rate: 33.3%). We speculated that the refractory cases (patients 18 and 19) showed widespread mucosal exfoliation in colo-

<table>
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<tr>
<th>Patient</th>
<th>Laboratory findings</th>
<th>Bloody stool</th>
<th>Preceding or concurrent ‘GVHD’</th>
<th>Endoscopic findings</th>
<th>Clinical diagnosis</th>
<th>Pathological diagnosis</th>
<th>Treatment</th>
<th>Response to therapy</th>
<th>Survival at 1 y after transplant</th>
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<tr>
<td>1</td>
<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD (mild) intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Possible TMA</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &gt; intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
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<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &gt; intestinal TMA (subtle)</td>
<td>Supportive therapy</td>
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<td>+</td>
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<tr>
<td>4</td>
<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &gt; intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &gt; intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &gt; intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
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<tr>
<td>7</td>
<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD + CMV + o</td>
<td>GVHD + CMV</td>
<td>GVHD &gt; intestinal TMA (subtle), CMV</td>
<td>Steroid, GCV</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>(−)</td>
<td>−</td>
<td>−</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &gt; intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
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<tr>
<td>9</td>
<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &lt; intestinal TMA (subtle)</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
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<tr>
<td>10</td>
<td>(−)</td>
<td>−</td>
<td>Skin, GVHD</td>
<td>GVHD</td>
<td>GVHD</td>
<td>GVHD &lt; intestinal TMA (subtle)</td>
<td>Steroid, support Tx</td>
<td>−</td>
<td>+</td>
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<tr>
<td>11</td>
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<td>Skin, liver + TMA, GVHD + GVHD</td>
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<td>Steroid† + Tacrolimus‡</td>
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<td>−</td>
<td></td>
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<tr>
<td>12</td>
<td>possible TMA</td>
<td>+</td>
<td>Skin, liver + TMA, GVHD + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Tacrolimus‡</td>
<td>+</td>
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<td>13</td>
<td>possible TMA</td>
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<td>Skin, liver + TMA, GVHD + GVHD</td>
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<td>Steroid + Cyclosporine A†</td>
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<td>+</td>
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<tr>
<td>14</td>
<td>possible TMA</td>
<td>−</td>
<td>TMA + CMV + GVHD + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Steroid + Tacrolimus‡ + GCV</td>
<td>+</td>
<td>+</td>
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<td>15</td>
<td>possible TMA</td>
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<td>TMA + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Tacrolimus‡ + Steroid, MMF</td>
<td>−</td>
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<tr>
<td>16</td>
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<td>−</td>
<td>Skin, GVHD + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Tacrolimus‡ + Steroid, MMF</td>
<td>−</td>
<td>−</td>
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<td>17</td>
<td>possible TMA</td>
<td>+</td>
<td>TMA + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Tacrolimus‡ + Steroid, MMF</td>
<td>−</td>
<td>−</td>
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<td>18</td>
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<td>TMA + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Tacrolimus‡</td>
<td>−</td>
<td>−</td>
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<td>19</td>
<td>possible TMA</td>
<td>−</td>
<td>TMA + GVHD</td>
<td>Mixed intestinal TMA (subtle)</td>
<td>Tacrolimus‡ + Steroid</td>
<td>−</td>
<td>+</td>
<td></td>
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</tr>
</tbody>
</table>

TMA, thrombotic microangiopathy; ‘GVHD, acute graft-versus-host disease; CMV, cytomegalovirus; Tx, therapy; MMF, mycophenolate mofetil; GCV, gancyclovir; MOF, multi-organ failure; DAD, diffuse alveolar damage.
Fig. 2. Characteristic colonoscopic and pathological findings for intestinal TMA and acute GVHD of the gut. 

(A) Colonoscopic findings of intestinal GVHD (a,b), mixed-type (c,d) and TMA (e,f). Mucosal edema and loss of transparency of vascular pattern were observed in GVHD (a), whereas, mucosal petechiae (c,e) and diffuse exfoliation (f) were observed in intestinal TMA. Indigo carmine stain was performed to visualize characteristic mucosal defects in GVHD(b,d). 

(B) Pathological findings of intestinal TMA (a,b) and GVHD (c) (HE stain). Microangiopathy with crypt loss (a: indicated by arrowheads) and interstitial hemorrhage (b) were observed in cases with intestinal TMA. Apoptotic epithelial cells with intraepithelial lymphocytes were observed in GVHD (c: indicated by arrowheads).
noscopy were already in the irreversible, advanced stage of intestinal TMA at the time of clinical diagnosis. Together with the high correlation between clinical and pathological results in the mixed type, we suggest that the colonoscopic findings of mucosal petechiae or diffuse exfoliation might be applicable criteria for the diagnosis of intestinal TMA or vascular damage.

Prior to the onset of colitis, 5 patients (patients 11, 12, 16, 17, and 18) were already administered steroid for some reasons, other than for GVHD treatment. It is noticeable that all 5 patients showed considerable ischemic changes. Paquette et al. (1998) suggested that the combined use of CyA and MTX and glucocorticoids as GVHD prophylaxis might predispose to the development of TMA by multivariate analysis. As steroid reportedly has adverse effects on vascular endothelial cells and platelets (Blajchman et al. 1979; Lewis et al. 1986; Yoshioka et al. 2007), we consider that steroid treatment might be also a risk factor of development of intestinal TMA.

Recently, Inamoto et al. (2009) reported that intensified immunosuppression before or after diarrhea following allogeneic stem cell transplantation increased non-relapse mortality, and suggested that avoiding intensified immunosuppression, which damages vascular endothelium, might improve transplant outcome. In our case series, the patients without mucosal petechiae or hemorrhage colonoscopically responded well to steroid administration (patients 1, 2, 4, 5, 6, 8, and 9). We suggest that especially the colonoscopic findings of mucosal petechiae or hemorrhage may reflect ischemic changes or intestinal TMA, and avoiding further vascular damages in those patients is important for improvement of transplant outcome.

Our analysis suggested that the clinical diagnosis of diarrhea after HSCT based on physical examination, laboratory data and colonoscopic findings was well correlated to the response to the therapy and the pathological diagnosis. As intestinal TMA could be a contributing cause of death when it develops to irreversible stage, an early clinical diagnosis of intestinal TMA, with the prompt initiation of appropriate therapies, might contribute to favorable results.

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Conflict of Interest
The authors have no conflict of interest to disclose.

References
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