Non-infectious gastrointestinal complications of GvHD

Grzegorz W. Basak
Dept. of Hematology, Oncology and Internal Diseases
The Medical University of Warsaw, Poland
Secretary of the Complications and Quality of Life Working Party, EBMT

Barcelona, 01.11.2013
Major points

- Basic symptoms of GI aGvHD and cGvHD;
- Diagnosis of GI GvHD (radiological studies, endoscopy, histology) and scoring
- Treatment: current and future approaches (standard systemic and local treatment, homing to gut, role of bacterial microbiota, regenerative therapies, nutritional support)
GI symptoms of acute and chronic GvHD

**Acute GvHD:**

<table>
<thead>
<tr>
<th>Upper GI tract</th>
<th>Nausea and/or anorexia PLUS positive histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower GI tract</td>
<td>Watery diarrhea &gt; 500ml +/- severe abdominal pain +/- bloody diarrhea or ileus (after exclusion of infectious etiology)</td>
</tr>
</tbody>
</table>

**Chronic GvHD:**

<table>
<thead>
<tr>
<th>Organ or Site</th>
<th>Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)</th>
<th>Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)</th>
<th>Other Features*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI tract</td>
<td>Esophageal web</td>
<td>Exocrine pancreatic insufficiency</td>
<td>Anorexia</td>
</tr>
<tr>
<td></td>
<td>Strictures or stenosis in the upper to mid third of the esophagus†</td>
<td></td>
<td>Nausea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vomiting</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diarrhea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weight loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Failure to thrive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(infants and children)</td>
</tr>
</tbody>
</table>

*Other features may include but are not limited to: rash, arthritis, liver or lung disease, ocular inflammation, or muscle weakness.
GI GvHD: diagnosis

- **aGvHD**: based on clinical symptoms confirmed by histologic biopsy (but treatment should be initiated ASAP, when required);
- **cGvHD**: only esophageal strictures or web (seen in gastroscopy or barium meal) are diagnostic (without biopsy). Other symptoms only with diagnostic or distinctive signs from other sites
Staging and grading of aGvHD (Glucksberg criteria) – focus on GI symptoms

<table>
<thead>
<tr>
<th>Stage</th>
<th>GI tract based on quantity of diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500-1000 ml</td>
</tr>
<tr>
<td>2</td>
<td>1000-1500 ml</td>
</tr>
<tr>
<td>3</td>
<td>&gt;1500 ml</td>
</tr>
<tr>
<td>4</td>
<td>Severe abdominal pain with/without ileus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>Grading of aGvHD depending on GI symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No GI symptoms</td>
</tr>
<tr>
<td>II</td>
<td>GI 1</td>
</tr>
<tr>
<td>III</td>
<td>GI 2-3</td>
</tr>
<tr>
<td>IV</td>
<td>GI 2-4</td>
</tr>
</tbody>
</table>

Severe GVHD carries a poor prognosis, with 25% long term survival for grade III and 5% for grade IV.
Scoring and grading of cGvHD – focus on GI symptoms

Mild chronic GVHD: only 1 or 2 organs or sites (except the lung), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites).

Moderate chronic GVHD: at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) (...)

Severe chronic GVHD: major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD.
Lower GI tract involvement in cGvHD has stronger impact on OS and NRM

In multivariate analysis lower GI involvement (HR, 1.67; P = .05) was associated with OS and NRM.

Any esophageal involvement and GI score greater than zero were associated with both symptoms and QOL with no consistent evidence that upper GI involvement add prognostic value for survival, overall symptom burden, or QOL.

Bowel ultrasonography detected either bowel wall thickness of the ileum and the colon or dilation in 16/17 patients and showed:

- 94% sensitivity,
- 95% specificity,
- 94.5% accuracy.


**Fig. 4.** Panels A–D show small and large bowel involvement assessed by bowel ultrasonography of a 45-year old female with gastrointestinal acute graft versus host disease. In panel A longitudinal scan of the terminal ileum performed with a convex probe shows bowel wall thickness at this level (white arrows). In panel B longitudinal scan at the same level with a linear probe shows details of the wall layers with normal lumen diameter (white arrowheads). In panels C and D longitudinal scan show dilation (5 cm) of the ascending and transverse colon (white arrowheads).

<table>
<thead>
<tr>
<th>Increased bowel wall thickening distribution in patients with and without acute gastrointestinal graft versus host disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Gl aGvHD N = 17 (%)</td>
</tr>
<tr>
<td>No Gl aGvHD N = 20 (%)</td>
</tr>
</tbody>
</table>
CT scan: thickening of bowell wall

**Figure 2.** Small and large bowel involvement. A 41-year-old man with clinical Grade IV graft-vs-host disease (GVHD). Patient died of GVHD. Unenhanced scan shows bowel wall thickening (thin white arrows), more severe in the large bowel (long arrow). Orally ingested gastrografin is present in the renal pelvis on both sides (thick white arrows).

**Figure 3.** Gastric involvement. A 24-year-old woman with clinical Grade IV graft-vs-host disease. Patient died from relapse of underlying disease, non-Hodgkin’s lymphoma. Unenhanced study shows gastric wall oedema and thickening (thick black arrow). Contrast material is seen in the renal pelvis on the right (thick white arrow). Thickening of gall bladder wall is seen as well (thin black arrow).

Shimoni et al. The British Journal of Radiology 2012;85:416
CT: Intestinal and extra-intestinal involvement

- Large bowel wall thickening and marked mucosal enhancement
- Gallbladder wall thickening
- Periportal oedema

Shimoni et al. The British Journal of Radiology 2012;85:416
Prognostic impact of findings in CT

- Diffuse small-bowel thickening and any involvement of the large bowel associated with severe clinical presentation.
- **Diffuse small-bowel disease correlates with poor prognosis.**
  - 8 of 21 patients responded to therapy, compared with 15 of 20 patients with other patterns (p=0.02)
  - the CI of GVHD-related death 62% and 24%(p=0.01).
  - Overall survival not significantly different.
  - Colonic disease correlated with severity of GVHD (p=0.04), but not with response to therapy or prognosis (p=0.45).

Shimoni et al. The British Journal of Radiology 2012;85:416
Role of PET/CT in diagnosis of GI GvHD

- Sensitivity 81%, specificity 90%, positive predictive value 60%, negative predictive value 96% and accuracy 83% for the diagnosis of acute GI-GVHD
- no significant differences of SUVmax values between grade 1–2 GI-GVHD and severe grade 3–4 GI-GVHD.
- noninvasive 18F-FDG PET/CT could become a valuable examination to be performed shortly before endoscopy to map acute GI-GVHD lesions, guide the biopsy sites and choose the appropriate endoscopic procedure, especially in those asymptomatic patients with a positive 18F-FDG PET/CT.

Bodet-Milin et al. BMT 2013 (in press)
Endoscopic studies of GI GvHD

• Endoscopy is usually used as a tool to obtain histologic biopsies, independently on macroscopic findings;

• Macroscopic lesions do not correlate well with histopathologic findings (Ross et al. Curr Opin Gastroenterol 2005;21:64)

• Visible endoscopic lesions are found in minority of cases (16-32%) at some institutions (Khan et al. Gastrointest Endosc 2006;64:379)

• Although the clinical grading of aGvHD seems to be the most relevant, there have been also approaches to provide criteria for endoscopic diagnosis and grading of GI GvHD (e.g. “Freiburg criteria” /Kreisel W et al. Eur J Gastroenterol Hepatol 1994; 6:723/).
Table 2: Macroscopic grading of aGVHD in the terminal ileum and colon: the ‘Freiburg Criteria’ for macroscopic diagnosis of intestinal aGVHD

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No clear-cut criteria. It suffices to state that there is no GVHD grade ( \geq 2 ). Cutoff between watch-and-wait and immediate therapy</td>
</tr>
<tr>
<td>2</td>
<td>Spotted erythema, initial aphthous lesions</td>
</tr>
<tr>
<td>3</td>
<td>Aphthous lesions (Crohn-like) or focal erosions</td>
</tr>
<tr>
<td>4</td>
<td>Confluent defects, ulcerations, complete denudation of the mucosa</td>
</tr>
</tbody>
</table>
Optimal site of GI biopsy

• The most common practice is to perform rectoscopy and/or gastroscopy with biopsies, depending on most prominent symptoms (from upper or lower GI)
  – Frequently without macroscopic findings, but features of GvHD in histology
  – Discordance between biopsy specimens from upper and lower GI tract in up to 45% of cases (Nydegger Pediatr Blood Cancer 2007;48:561)
  – Some studies indicate that biopsy from stomach is more likely to show GvHD and the others that from distal colon;

• Rectal biopsy seems to be the easiest and safest although up to 38% of GvHD cases might be missed.
  – No need to perform sigmoid or more proximal colon (concordant results with rectum) (?).

• If biopsy from rectum negative and symptoms persist, biopsy from stomach or duodenum advocated.

• Increased risk of bleeding after duodenal biopsies in children after HSCT (Khan et al. Gastrointest Endosc 2006;64:379)
Optimal site of GI biopsy – alternative strategy:

• Colonoscopy in conjunction with inspection of the terminal ileum may have higher diagnostic yield (100%) as a combination of rectosigmoidoscopy and gastro-duodenoscopy (92%).

• If the macroscopic aspect of the colon is typical for aGVHD, an ileoscopy need not be done.

• If the colon appears macroscopically normal the terminal ileum must be inspected.
  – An endoscopic examination of the upper GIT may then be omitted.

/Kreisel et al Bone Marrow Transplantation 2012; 47: 430/
Histology of GI GvHD

• Characteristic changes for acute GI GvHD: epithelial cell apoptosis evolving to “exploding crypt” cells

• No histologic changes in the GI tract that are specific for chronic GvHD (all changes observed are sequellae of refractory acute or late acute GvHD)

Crypt cell apoptosis may be present also in many other situations: protein pump inhibitors, MMF, CMV or Cryptosporidium, preparatory regimen for colonoscopy....
Recommendations for final diagnosis categories for biopsy reporting from the NIH Consensus Project

<table>
<thead>
<tr>
<th>category</th>
<th>explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>not GVHD</td>
<td>for biopsies with no evidence of GVHD</td>
</tr>
<tr>
<td>possible GVHD</td>
<td>evidence of GVHD but other possible explanation for findings (CMV with apoptotic bodies found only near CMV inclusions, MMF-associated colitis, or other clinical features that suggest or favor a drug reaction)</td>
</tr>
</tbody>
</table>
| consistent with GVHD     | Clear histologic evidence of GVHD but with mitigating factors e.g.:  
  • limited sample;  
  • minimal findings such as single or rare apoptotic epithelial cells with no alternative explanation;  
  • recent chemotherapy or radiotherapy.  
  • biopsy with CMV but with abundant apoptosis not associated with CMV-infected cells as identified by immunohistochemistry |
| GVHD                      | unequivocal evidence of GVHD                                                                                                                |
• Patients with GI biopsies interpreted as “negative for GVHD” or “possible GVHD” may not necessarily be treated for GVHD, depending upon other clinical factors, although given the biopsy false-negative rate, treatment may be considered if other causes for symptoms or endoscopic findings are not found in the biopsy.

• Patients with biopsy results interpreted as “consistent with GVHD” or “GVHD” will generally be treated with increased immunosuppression.
Histologic grading of GI aGvHD

**Grade 1:** Isolated apoptotic epithelial cells without crypt loss

**Grade 2:** Loss of isolated crypts without the loss of contiguous crypts

**Grade 3:** Loss of 2 or more contiguous crypts

**Grade 4:** Extensive crypt loss with mucosal denudation

Histologic grading – why not commonly used?

- Controversial
- May reflect the timing of the biopsy in relation to onset of GvHD, duration of activity, influence of immunosuppression on the degree of inflammation and adequacy of the sample;
- Not validated to predict refractoriness or other relevant end points;
- Histologic grade of GI GvHD does not correspond with endoscopic findings or clinical features (patchy distribution of lesions in GI tract)
- Severe crypt loss may correspond to higher stool volumes and refractoriness to treatment (Melson et al. Am J Hematol 2007;82:881)
- Low interobserver agreement
Loss of Paneth cells as an indicator of severity and prognostic risk factor

GI GvHD biomarkers: calprotectin (1)

Fecal calprotectin level (mg/kg) is significantly higher in GI aGvHD than in relevant control groups (aGvHD with other organ involvement, infective enteritis, diarrhea after autologous SCT)

Difference less remarkable in case of GI cGvHD

GI GvHD biomarkers: **calprotectin** (2)

stratification of patients with GI GvHD by calprotectin level

The survival rate by GI-GVHD stage and calprotectin levels with 3 groups:
1. stage 1 and stage 2 with a normal concentration of calprotectin;
2. stage 2 with a high concentration of calprotectin and stage 3 with a normal concentration of calprotectin;
3. stage 3 with a high concentration of calprotectin.

Prophylaxis and treatment of GVHD: EBMT–ELN working group recommendations for a standardized practice

T Ruutu¹, A Gratwohl², T de Witte³, B Afanasyev⁴, J Apperley⁵, A Bacigalupo⁶, F Dazzi⁷, P Dreger⁸, R Duarte⁹, J Finke¹⁰, L Garderet¹¹, H Greinin¹², E Holler¹³, N Kröger¹⁴, A Lawitschka¹⁵, M Mothy¹⁶, A Nagler¹⁷, J Passweg¹⁸, O Ringdén¹⁹, G Socie²⁰, J Sierra²¹, A Sureda²², W Wiktor-Jedrzejczak²³, A Madrigal²⁴ and D Niederwieser²⁵, a working group of the European Group for Blood and Marrow Transplantation (EBMT) and the European LeukemiaNet (ELN)

Treatment of GVHD

Treatment of acute GVHD

First-line treatment

The first-line treatment of acute GVHD is MP.

Treatment is initiated for acute GVHD of grade II or higher.

The initial MP dose is 2 mg/kg/day.

MP is given in two divided doses per day.

The initial dose is continued for 7 days. Treatment can be changed in case of clear progression after 5 days, but there is no evidence that change in treatment will affect the outcome.

No reduction of the dose is done during the first 7 days.

Tapering of the dose is done slowly and depending on the response. No marked dose reductions are done in the early phase. MP is not discontinued before all signs of GVHD have disappeared.

Non-absorbable oral steroid (budesonide) is given, along with systemic corticosteroid, for GI GVHD in the dose of 9 mg/kg/day in one daily dose p.o.

Topical steroids are used for skin GVHD according to centre policy.

The decision to initiate treatment is based on clinical signs. Skin biopsy before initiation of treatment is recommended, but the decision to treat should not depend on the biopsy result. The same recommendation applies to upper GI or sigmoid biopsy if GI manifestation is suspected.

Second-line treatment

The indication for second-line treatment is failure of MP treatment as defined above.

There is no standard second-line treatment for acute GVHD. Widely used components are MMF, anti-TNF-Abs, other MoAbs, ATG, extracorporeal photopheresis, MTX and mesenchymal stem cells. Continuation of calcineurin inhibitors and corticosteroids with optimal supportive care is considered a valid option. Centres should have and follow their institutional guidelines, and the patients should be treated in trials as far as possible.

Treatment of chronic GVHD

Indication for starting treatment of chronic GVHD depends on the type and severity of symptoms and the speed of symptom progression in the context of other relevant variables, such as disease risk, chimerism, and minimal residual disease results.

Evaluation of chronic GVHD according to the NIH consensus guidelines is recommended.

The first-line treatment of newly diagnosed chronic GVHD in patients not on any immunosuppressive drug, or receiving CsA (or tacrolimus) only, is corticosteroid.

If the patient is already on corticosteroid treatment (for example, following treatment of acute GVHD), CsA is added to the treatment and the dose of corticosteroid is increased.

If the patient is already receiving corticosteroid and CsA at the time of the onset of chronic GVHD, no standard treatment is available. Continuation of corticosteroid and CsA with optimal supportive measures is a valid option. Alternatively, the patient should be treated in a clinical trial if possible.

The time needed to preliminarily assess the efficacy of the first-line treatment of chronic GVHD is at least 1 month.

There is no standard second-line treatment for chronic GVHD. The most widely used components of second-line treatment, in addition to corticosteroids, are extracorporeal photopheresis, MMF, rituximab, calcineurin inhibitors and mTOR inhibitors. Centres should have and follow their institutional guidelines, and the patients should be treated in trials as far as possible.
Next-line treatments directed towards GI GvHD

- Mesalazine, sulphasalazine;
- Etanercept;
- Other systemic treatments

Supportive care for GI GvHD

- Critical
- gut rest, hyperalimentation, fluid and electrolyte repletion.
Nutritional support in patients with GI GVHD

- GI GVHD is often associated with malnutrition, protein losing enteropathy, magnesium derangements, and deficiencies of zinc, vitamin B12 and vitamin D.
- Limited evidence exists on derangements of magnesium, resting energy expenditure, bone mineral density and pancreatic function, and some beneficial effects of n-3 polyunsaturated fatty acids and pancreatic enzyme replacement therapy.

/van der Meij et al. Bone Marrow Transplantation 2013;48:474/
Nutritional support in patients with GVHD of the digestive tract

• Expert opinions recommend:
  – Adequate amounts of energy, at least 1.5 g protein/kg body weight, supplied by total parenteral nutrition in cases of severe diarrhoea.
  – When diarrhoea is <500 mL a day, a stepwise oral upgrade diet can be followed.

• No studies exist on probiotics, prebiotics, dietary fibre and immunonutrition in GVHD-DT patients.

• Future research should focus on absorption capacity, vitamin and mineral status, and nutritional support strategies.

/van der Meij et al. Bone Marrow Transplantation (2013) 48, 474/
Emerging therapies for GI GvHD

• Manipulation of gut homing properties of T lymphocytes
• Manipulation of gut flora
• Regenerative approaches
Beta(7) integrin as a therapeutic target in GI GvHD

- Expression of \textit{alpha(4)beta(7) integrin} on donor T cells (binds to MAdCAM, which is present on high endothelial venules of mucosal lymphoid organs) is involved in gut GvHD /Petrovic A et al. Blood. 2004;103:1542-7/

- Presence of either \textbf{L-selectin or alpha4beta7 integrin} is required for donor CD4+ T cell homing to mesenteric lymph nodes and Peyer’s patches. Inhibition of \textbf{BOTH} of them was required to significantly reduce incidence of GvHD colitis /Dutt S et al. Blood 2005; 106: 4009/
Deprivation of retinoic acid decreases T cell homing capacity to the gut and symptoms of GI GvHD

• Expression of the gut-specific homing receptors integrin-α4β7 and chemokine receptor CCR9 on T cells is imprinted in gut-associated lymphoid tissues (GALT) under the influence of the vitamin A metabolite retinoic acid.

• Expression of integrin-α4β7 and CCR9 in GALT was decreased in mice fed with vit. A-deprived diet;

• In these mice, homing capacity to the gut was decreased and extent of GI GvHD was ameliorated, that translated into prolonged survival;

• All these mice died because lymphocytes homed and attacked other organs instead of GI system.

Koenecke C. PLOS One 2012; 7: e38252
Inhibition of RARα signaling as a therapeutic target in GI GvHD

- Increasing RAR signaling accelerated GVHD lethality, whereas donor T cells expressing a dominant-negative RARα (dnRARα) showed markedly diminished lethality.

- The dnRARα transgenic T cells showed reduced Th1 differentiation and α4β7 and CCR9 expression associated with poor intestinal migration, low GVHD pathology, and reduced intestinal permeability, primarily via CD4+ T cells.

- The inhibition of RAR signaling augmented donor-induced Treg generation and expansion in vivo, while preserving graft-versus-leukemia effects.

Modulation of gut microbiome for the prophylaxis and treatment of GvHD


• Clinical consequence: decontamination of GI system decreases probability of GvHD

• Ciprofloxacin alone vs. ciprofloxacin + metronidazole (50% vs. 25% aGvHD grade 2-4, p=0.002)

Modulation of gut microbiome for the prophylaxis and treatment of GvHD

- α-defensins produced by Paneth cells in the gut selectively kill noncommensals while protecting commensal bacteria;
- Paneth cells are targeted by GvHD – lack of defensin secretion causes loss of physiologic diversity of gut bacteria with overwhelming expansion of E. coli species;
- A significant correlation between alteration in the intestinal microbiota and GVHD severity;
- Oral administration of polymyxin B inhibited outgrowth of E. coli and ameliorated GVHD;
- Occurrence of GI GvHD may therefore increase chance of E. coli septicaemia, but the induced changes in gut flora may further exacerbate GI GvHD;
- The gut flora has important impact on GvHD and its MODULATION (not only decontamination) may play a role in GvHD prophylaxis and treatment;

Regenerative approaches

• Prophylactic treatment of mice with the rhu Keratinocyte Growth Factor (KGF) protected from apoptosis of intestinal epithelial cells, LPS mediated TNF release, and finally lethal GvHD while maintaining GvL effects /Ellison et al. Journal of Clinical Immunology 2004;24:197/.

• However, in a randomized clinical trial KGF reduced severity of mucositis in patients receiving TBI but did not affect GI GvHD and outcome as expected from murine data /Blazar et al. Blood 2006;108:3216; Levine et al. BBMT 2008;14:1017/

• FUTURE: How to enhance gut regeneration after successful inhibition of gut inflammatory response?