Department of Clinical Science, Intervention and Technology,
Division of Pediatrics
Karolinska Institutet, Stockholm, Sweden

# ACUTE GASTROINTESTINAL GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTED CHILDREN AND ADOLESCENTS

**-** CLINICAL ASPECTS OF HISTOPATHOLOGICAL EVALUATION AND RISK FACTORS

Thomas Mårtensson


Karolinska Institutet

Stockholm 2020

Published by Karolinska Institutet.
Printed by AJ E-Print AB

ISBN 978-91-7831-779-0

# ACUTE GASTROINTESTINAL GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTED CHILDREN AND ADOLESCENTS – CLINICAL ASPECTS OF HISTOPATHOLOGICAL EVALUATION AND RISK FACTORS

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

This PhD thesis will be publicly defended, October the 16th, 2020, at 09:30, in lecture hall 4U, Solen, Alfred Nobels Allé 8, Campus Flemingsberg

Zoom link: https://ki-se.zoom.us/j/3312967722

By

**Thomas Mårtensson**

*Principal Supervisor:*
Professor, Britt Gustafsson
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Pediatrics

*Co-supervisors:*
Associate Professor, Thomas H. Casswall
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Pediatrics

Professor, Moustapha Hassan
Karolinska Institutet
Department of Laboratory Medicine and Clinical Research Centre

Professor, Jonas Mattsson
Karolinska Institutet
Department of Oncology-Pathology

*Opponent:*
Associate Professor, Kristina Carlsson
Uppsala University
Department of Medical Sciences, Haematology

*Examination Board:*
Associate Professor, Jukka Vakkila
University of Helsinki, Finland
Haematology Research Unit

Associate Professor, Robert Saalman
University of Gothenburg
Institute of Clinical Sciences
Department of Pediatrics

Associate professor, Johan Mölne
University of Gothenburg
Institute of Biomedicine
Department of Laboratory Medicine

# ABSTRACT

**Background**: Acute graft-versus-host disease (aGVHD), following allogeneic hematopoietic stem cell transplantation (HSCT), is a potentially life-threating condition. Gastrointestinal involvement of aGVHD (GI-aGVHD) affects approximately every fourth transplanted child. The diagnosis of GI-aGVHD is primarily symptom-based. However, symptoms associated with GI-aGVHD are nonspecific; thus, histopathological confirmation of the diagnosis, is recommended. The overall objectives of this thesis were: *i*) to evaluate the influence of two different conditioning regimens on the incidence of GI-aGVHD, and *ii*) to evaluate clinical aspects of the currently recommended diagnostic approach to GI-aGVHD, i.e., endoscopy-guided histopathological assessment, applied to pediatric HSCT patients.

**Patients and methods**: Four retrospective cohort studies were included in this thesis. *Paper I* enrolled all children with HSCT performed during 2000–2010 at Karolinska University Hospital Huddinge who also had underlying diagnoses of juvenile myelomonocytic leukemia (JMML) or myelodysplastic syndrome (MDS). The children were conditioned with busulfan (Bu) and cyclophosphamide (Cy), with or without addition of melphalan (Mel). *Paper II-IV* included all children who underwent HSCT at any of the four HSCT centers in Sweden between 2000 and 2012 and with endoscopy-guided histopathological assessment performed to confirm symptom-based GI-aGVHD within one-year post-HSCT. In *paper III-IV* a retrospective, blinded, histopathological assessment (RIHA) was carried out based on the *National Institutes of Health (NIH) 2014* criteria for histopathology-based GI-GVHD. *Paper IV* only included those with at least a biopsy sampling from the rectosigmoid area and the area proximal to the left colonic flexure.

**Results:** *Paper 1*: Twenty-five children were enrolled. Forty-seven percent (8/17) of the children that received addition of Mel to the BuCy conditioning, versus none (0/8) in the BuCy group, developed GI-aGVHD (stages 2-4) ($p<0.05$). *Paper II*: Based on 68 children with 91 endoscopic occasions, treatment changes in response to histopathology reports occurred in 48% (44/91). *Paper III*: Seventy children with 92 endoscopic occasions were assessed. Histopathology-based GI-GVHD diagnosis was established in 67 of 92 (73%) endoscopic occasions in the RIHA and in 50 of 92 (54%) in the clinical standard histopathological assessment ($p=0.014$). The risk of a subsequent re-endoscopy within one-year post-HSCT was higher in endoscopic occasions with GI-GVHD solely detected in RIHA versus non-GI-GVHD in both assessments ($p=0.005$). *Paper IV*: Forty-four children with 51 endoscopic occasions were analyzed. Biopsies from the rectosigmoid area had 85% sensitivity for RIHA-based GI-GVHD diagnosis. The corresponding figure for combined biopsy sampling from both rectosigmoid area and upper gastrointestinal tract was 97% and was similarly high compared with biopsies collected from complete lower endoscopy.

**Conclusions:** *I*) Addition of Mel to the BuCy conditioning increased the incidence of symptom-based GI-aGVHD in children with JMML and MDS. *II)* Endoscopy-guided histopathological assessment was found to influence the treatment decisions and should therefore be considered in children with GI-aGVHD. *III*) In children with symptom-based GI-aGVHD, without confirmation of the diagnosis by clinical standard histopathological assessment, a second histopathological assessment based on the *NIH 2014* criteria should be considered before performing a re-endoscopy. *IV*) Sigmoidoscopy combined with upper endoscopy, colonoscopy/ileocolonoscopy, or full upper and lower endoscopy should be considered as preferred choices for the endoscopic procedure in children with clinically suspected GI-aGVHD.

# LIST OF SCIENTIFIC PAPERS

This thesis is based on following papers:

I. **Mårtensson T**, Priftakis P, Casswall T, Ringdén O, Mattsson J, Remberger M, Hassan M*, Gustafsson B.* Increased risk of gastrointestinal acute GVHD following the addition of melphalan to busulfan/cyclophosphamide conditioning. *Pediatr Transplant.* 2013 May;17(3):285-93.

II. **Mårtensson T**, Mellgren K, Toporski J, Arvidson J, Szakos A, Casswall T. H.*, Gustafsson B.* Clinical relevance of endoscopy with histopathological assessment in children with suspected gastrointestinal graft-versus-host disease. *Clin Transplant.* 2020 Apr 5. [Epub ahead of print]

III. **Mårtensson T**, Szakos A, Mellgren K, Toporski J, Arvidson J, Mattsson J, Gustafsson B.*, Casswall T. H.*. Diagnostic disagreement between clinical standard histopathological-, and retrospective assessment of histopathology-based gastrointestinal Graft-Versus-Host Disease in children. Accepted for publication in *Pediatr Transplant* (July 23, 2020)

IV. **Mårtensson T,** Szakos A Mellgren K, Toporski J, Arvidson J, Casswall T. H.*, Gustafsson B.* Choice of Endoscopic Procedure in Children With Clinically Suspected Gastrointestinal Graft-versus-host Disease. *J Pediatr Gastroenterol Nutr.* 2018 May;66(5):744-750.

**Shared last authorship*

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| aGVHD/cGVHD | Acute/chronic graft-versus-host disease |
| APC | Antigen presenting cell |
| ATP | Adenosine triphosphate |
| ATG | Anti-thymocyte globulin |
| B-cell | Bone marrow derived lymphocyte |
| BSA | Body skin area |
| Bu | Busulfan |
| *C. difficile* | *Clostridium difficile* |
| CDI | *Clostridium difficile* infection |
| CIBMTR | The Center for International Blood and Marrow Transplant Research |
| CMV | Cytomegalovirus |
| CSHA | Clinical standard histopathological assessment |
| CT | Computed tomography |
| CTL | Cytotoxic effector T lymphocyte |
| Cy | Cyclophosphamide |
| EBMT | The European Society for Blood and Marrow Transplantation |
| EBV | Epstein-Barr virus |
| *E. coli* | *Escherichia coli* |
| EGD | Esophagogastroduodenoscopy |
| EO | Endoscopic occasion |
| FMT | Fecal microbiota transplantation |
| FoxP3 | Forkhead Box Protein 3 |
| GI | Gastrointestinal tract |
| GI-aGVHD | Gastrointestinal acute graft-versus-host disease (symptom-based) |
| GI-cGVHD | Gastrointestinal chronic graft-versus-host disease |
| GI-GVHD | Gastrointestinal graft-versus-host disease (histopathology-based) |
| GVT | Graft-versus-tumor |
| HLA | Human Leukocyte Antigen |

| | |
|---|---|
| HSCT | Allogeneic hematopoietic stem cell transplantation |
| IBD | Inflammatory bowel disease |
| IL | Interleukin |
| IFN-γ | Interferon-gamma |
| JAK | Janus-activated kinase |
| JMML | Juvenile myelomonocytic leukemia |
| LPS | Lipopolysaccharide |
| MAC | Myeloablative conditioning |
| MAGIC | The Mount Sinai Acute GVHD International Consortium |
| Mel | Melphalan |
| mHA | Minor histocompatibility antigen |
| MDS | Myelodysplastic syndrome |
| MRI | Magnetic resonance imaging |
| NIH | The National Institutes of Health |
| NK cell | Natural killer cell |
| PCR | Polymerase chain reaction |
| PDC | Pediatric Crohn disease |
| PTLD | Post-transplant lymphoproliferative disease |
| Reg-3-α | Regenerating islet-derived 3 alpha |
| RIC | Reduced intensity conditioning |
| SR-aGVHD | Steroid refractory acute graft-versus-host disease |
| Spp | Several species |
| RIHA | Retrospective, independent, histopathological assessment |
| T-cell | Thymus derived lymphocyte |
| Th cell | Helper T-cell |
| TLR | Toll-like receptor |
| TNF-α | Tumor necrosis factor alpha |
| T-reg | Regulatory T-cell |
| US | Ultrasonography |

# 1 GENERAL BACKGROUND

## 1.1 HISTORICAL OVERVIEW

### 1.1.1 Allogeneic hematopoietic stem cell transplantation

The era of allogeneic hematopoietic stem cell transplantation (HSCT) began in the 1950s when *Vos et al.* (1) and *Barnes et al.* (2) exposed mice to supra-lethal doses of irradiation, followed by intravenous infusion of bone marrow cells from mice of a different strain. *Barnes et al.* used mice with leukemia and showed that the malignancy was most often eradicated by this procedure. However, in both studies, the majority of the animals experienced a "delayed" death that was preceded by profuse diarrhea and reduced body weight (1, 2). The observed syndrome was called the *secondary disease*, later renamed graft-versus-host disease (GVHD).

GVHD was studied in depth by *van Bekkum et al.*, who described GVHD as a multiorgan failure, affecting the skin, gastrointestinal tract and liver (3). A breakthrough in creating the prerequisites for introduction of HSCT in clinical practice came in the mid–late 1960s with the discovery of Human Leukocyte Antigens (HLA), the major trigger of alloreactivity, and the development of drug regimens to protect against and treat GVHD.

In 1968, reports in *The Lancet* described the first two children treated with HSCT (4, 5). Seven years later, Huddinge Hospital/Karolinska University Hospital Huddinge performed the first HSCT in a Swedish child. Within a few years, HSCT became an established treatment procedure for children in Sweden.

### 1.1.2 Pediatric gastrointestinal endoscopy

The first endoscopy-like instrument, the *lichtleiter*, was introduced by the German physician Philip Bozzini in 1805 (6). The instrument was rigid and used candlelight for illumination. Its rigidity and poor illumination constrained the use of the *lichtleiter* in clinical practice. Modern, fully flexible endoscopes were introduced in 1957 and flexible GI endoscopes for children appeared in clinical practice in the 1970s (6).

The first pediatric endoscopy for diagnostic of GVHD in the gastrointestinal tract was performed in 1968 (5). A five-month-old boy with sex-linked lymphopenic immunological deficiency underwent HSCT, with his eight-year-old sister as the stem cell donor. At approximately two weeks post-HSCT, the boy developed bloody diarrhea. During proctoscopy with biopsy sampling, small mucosal ulcers were observed. The subsequent histopathological assessment of collected biopsies revealed crypt lesions, moderate mononuclear cell infiltration, and increased numbers of mast cells and eosinophils. The histopathological pattern was interpreted as gastrointestinal GVHD. Fortunately, the boy recovered and was discharged from the hospital at two months post-HSCT.

Today, endoscopy with biopsy sampling, followed by histopathological assessment, is the overall recommended diagnostic procedure to attribute GI symptoms that develop post-HSCT to gastrointestinal GVHD (7-9).

## 1.2 INDICATION OF PEDIATRIC ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Approximately 40–50 children under 18 years of age undergo HSCT annually in Sweden. Roughly 50 % of the HSCTs are performed due to malignant blood diseases, with the most common being acute lymphoblastic leukemia, followed by acute myeloid leukemia (10, 11). The remaining 50 % represents a spectrum of non-malignant diseases, such as hemoglobinopathies, bone marrow failure, severe immune deficiencies, and metabolic diseases (10, 12).

## 1.3 TRANSPLANTATION PROCEDURE

### 1.3.1 Considerations during the pre-HSCT process

#### *1.3.1.1 Donor*

A HLA mismatch between stem cell donor and the recipient is the most substantial risk factor for the development of GVHD (13-15). Siblings, followed by parents, are first investigated as potential donors. As the second source, a potential donor is searched through international live donor registries, such as *the National Marrow Donor Program* and *the Tobias Registry* or through accredited public umbilical cord blood banks. The selection of a donor is primarily based on the best possible HLA matching between recipient and donor, with preference for a matched sibling donor (16). Beyond HLA compatibility, serological-based matching of cytomegalovirus (CMV), Epstein-Barr virus (EBV) is preferred, and furthermore, a younger donor is preferred compared with an older one, as well as avoidance of female-donor-to-male-recipient transplantation (16).

#### *1.3.1.2 Stem cell source*

Potential stem cell sources are bone marrow, peripheral blood stem cells, and umbilical cord blood. The choice of stem cell source, if several are available, is based on the probability that each might increase the risk of GVHD, relapse (i.e., recurrence of the underlying disease that was the indication for the transplantation), and graft failure (16, 17). The choice of stem cell source also depends on the underlying diagnosis of HSCT and the donor preference of donation method (16). In children, bone marrow is the most frequently used stem cell source, whereas it is peripheral blood stem cells in adults (18).

#### *1.3.1.3 Conditioning regimen*

The conditioning or preparative regimen refers to chemotherapy with or without simultaneous irradiation administered before the infusion of the graft. The conditioning regimen induces immunosuppression in the recipient, thereby enabling engraftment, thus avoiding rejection of the graft (19). The conditioning regimen also provides an antitumor effect (19).

The two main categories of conditioning regimen are myeloablative conditioning (MAC) and reduced intensity conditioning (RIC). MAC is an extensive conditioning regimen and was previously regarded as the gold standard if the indication for HSCT was malignant blood disease. However, growing evidence now indicates that RIC can be used instead of MAC, since eradication of malignant cells is more related to the so-called graft-versus-tumor (GVT) effect than to the intensity of the conditioning regimen (20).

#### *1.3.1.4 GVHD prophylaxis*

GVHD prophylaxis consists of immunosuppressive drugs to reduce the risk of development of GVHD. The thymus-derived lymphocytes (T-cells) of the graft are the target cells of this prophylaxis. The choice of prophylaxis is based on an integrated analysis of each patient regarding the occurrence of factors that may increase the risk of GVHD. The dosage and duration of the prophylaxis depends on whether a malignant or a non-malignant disease is the underlying diagnosis for the HSCT. If the underlying diagnosis is a non-malignant disease, no GVT effect is needed; therefore, higher doses and a longer duration of the prophylaxis are preferred.

In children, the standard GVHD prophylaxis is cyclosporine A, combined with a short course of methotrexate (21). Polyclonal anti-thymocyte globulin (ATG) is most often added if the risk of GVHD is elevated, such as in cases with a HLA-matched unrelated donor or a HLA mismatched donor (19, 21). In a high-risk situation for GVHD, such as in haploidentical stem cell transplantation (stem cells from one of the parents), the addition of two doses of cyclophosphamide post-HSCT (PT-Cy) might be an option (22).

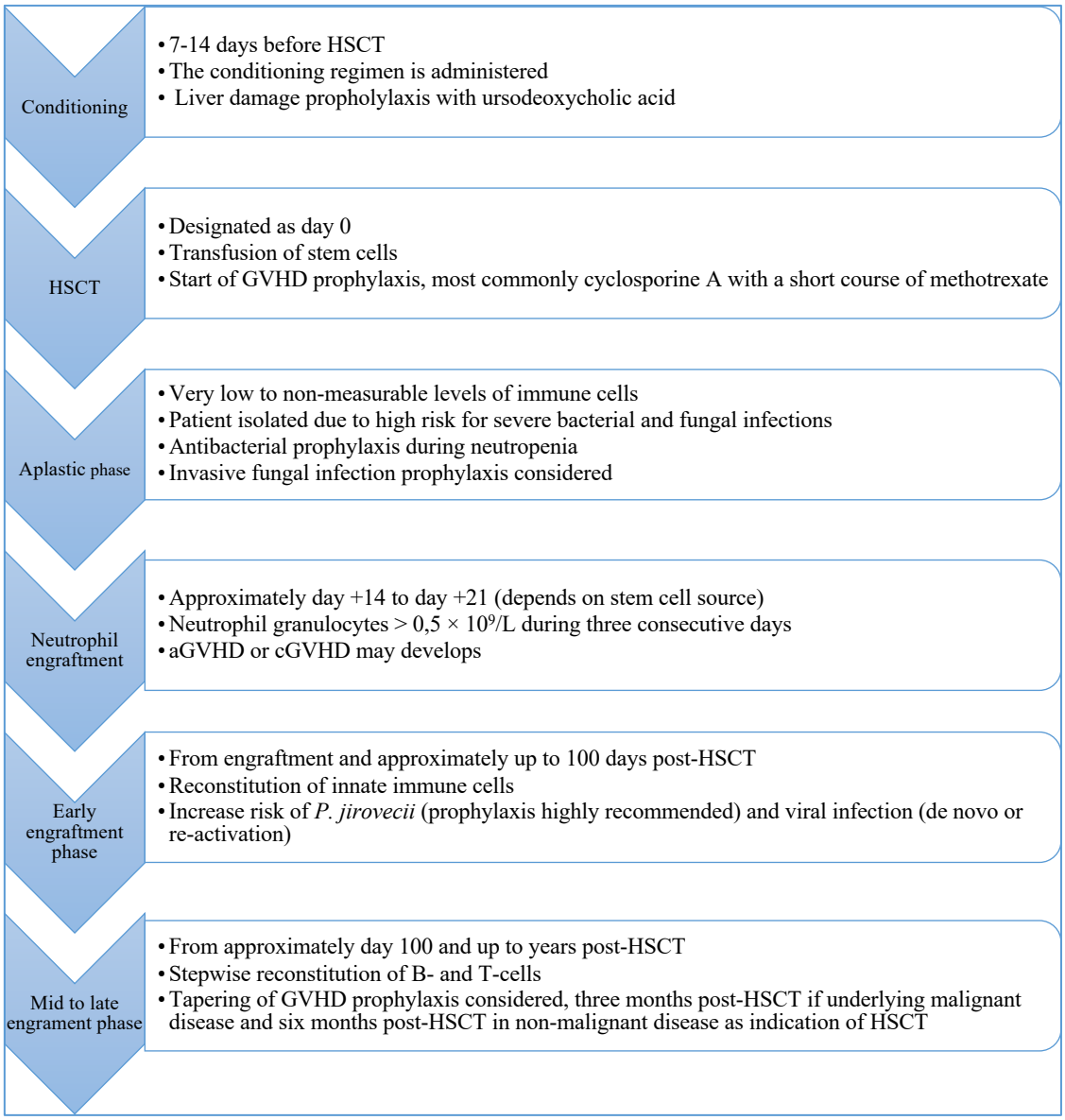


**Figure 1**. Schematic overview of the HSCT process (21, 23-25)

### 1.3.2 The HSCT process

A schematic overview of the HSCT process is presented in figure 1.

#### *1.3.2.1 Engraftment*

Engraftment indicates that the graft has survived and has started to produce new hematological cells. Neutrophil engraftment is defined as absolute neutrophil count of > 0.5 × $10^9$ cells/L over three consecutive days, whereas platelet engraftment is defined as levels of platelets of >20 × $10^9$ cells/L over seven consecutive days (26).

Engraftment occurs most frequently at two weeks after the transplantation if peripheral blood stem cells are used and approximately three weeks post-HSCT in bone marrow transplantation (27). Engraftment indicates the time point at which the clinical onset of GVHD may occur (23).

## 1.4 ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE

The *National Institutes of Health (NIH)* classifies GVHD into two entities: acute GVHD (aGVHD) and chronic GVHD (cGVHD) (28). However, in daily clinical practice, the distinction between these two is sometimes problematic. Furthermore, overlap GVHD, a subgroup of cGVHD with features of both acute and chronic GVHD occurring simultaneously, is a defined subgroup (28).

### 1.4.1 Acute GVHD

Acute GVHD is primarily a symptom-based diagnosis when it affects the skin or the GI tract. In acute liver–GVHD, the diagnosis is based on elevated serum bilirubin (Table 1). In classical aGVHD, the onset of symptoms occurs within 100 days post HSCT (29). In late onset and persistent aGVHD, the onset of symptoms is observed after, or persists beyond, 100 days post HSCT (29).

**Table 1.** Staging of acute GVHD in children (bodyweight < 50 kg) and in adolescents or adults (bodyweight >50 kg), in accordance with *The Mount Sinai Acute GVHD International Consortium*[#]

| Stage | Skin | Liver[A] | Upper GI tract | Lower GI tract |
|---|---|---|---|---|
| 0 | No active GVHD rash | < 34 µmol/L | | |
| 1 | Maculopapular rash[B] <25% BSA | 34-50 µmol/L | Persistent nausea[C], vomiting[D] or anorexia with weight loss | <*50 kg*, diarrhea[E] 10-19.9 mL/kg/day or 4-6 episodes/day >*50 kg*, diarrhea[E] 500-999 mL/day or 3-4 episodes/day |
| 2 | Maculopapular rash [B] 25-50% BSA | 51-102 µmol/L | | <*50 kg*, diarrhea[E] 20-30 mL/kg/day or 7-10 episodes/day >*50 kg*, diarrhea[E] 1000-1500 mL/day or 5-7 episodes/day |
| 3 | Maculopapular rash[B] >50% BSA | 103-255 µmol/L | | < *50 kg*, diarrhea[E] >30 mL/kg/day or >10 episodes/day >*50 kg*, diarrhea[E] >1500 mL/day or >7 episodes/day |
| 4 | Erythroderma[B] >50% BSA and bullous formation and desquamation >5% BSA | > 255 µmol/L | | Severe abdominal pain[F] with or without ileus or grossly bloody stool (regardless of stool volume) |

[A]Hyperbilirubinemia developed simultaneously or after onset of acute GVHD in the skin or in the GI tract, and non-GVHD causes of hyperbilirubinemia ruled out with reasonable certainty. [B]Hyperpigmentation excluded.
[C]At least during three consecutive days. [D]At least two vomiting episodes during two consecutive days.
[E]Frequencies of defecations or volumes of diarrheas (preferably) calculated as an average during three consecutive days. [F]Substantial impact of the performance status and requiring initiating of high doses of narcotic pain relief medication or substantial dose escalating, if ongoing medication at onset of stage IV acute GVHD in lower GI tract.
[#]Published with kindly permission of *The Mount Sinai Acute GVHD International Consortium (Harris et al, Biol Blood Marrow Transplant 2016)*
(GVHD: Graft-versus-host disease, BSA: Body skin area, GI: Gastrointestinal tract)

### *1.4.1.1 Severity staging and grading*

A disease severity scoring system for aGVHD was developed in the 1970s (30). It was modified in 1994 to the so-called modified Glucksberg or Keystone criteria (31), followed by further minor revisions. Today, several different scoring systems exist. However, in the context of clinical aGVHD research, *The European Society for Blood and Marrow Transplantation (EBMT), The NIH and The Center for International Blood and Marrow Transplant Research (CIBMTR)*, recommend the aGVHD staging/grading system of *The Mount Sinai Acute GVHD International Consortium (MAGIC)* (32).

The scoring of aGVHD is based on the disease severity in the skin, liver, and GI tract (Table 1). The disease stage (0–4) in each affected organ is used to provide an overall disease severity grade (1–4) (Table 2). The overall disease severity grade corresponds inversely to the probability of survival. Roughly, the higher overall disease severity grade, the lower probability of survival (33).

**Table 2.** Overall grading of acute graft-versus-host disease in accordance with *The Mount Sinai Acute GVHD International Consortium*[#]

| Grade | Skin | | Liver | | Upper GI tract | | Lower GI tract |
|---|---|---|---|---|---|---|---|
| 0 | - | | - | | - | | - |
| 1 | Stage 1-2 | | - | | - | | - |
| 2 | Stage 3 | *and/or* | Stage 1 | *and/or* | Stage 1 | *and/or* | Stage 1 |
| 3 | | | Stage 2-3 | | | *and/or* | Stage 2-3 |
| 4 | Stage 4 | *and/or* | Stage 4 | | | *and/or* | Stage 4 |

[#]Published with kindly permission of *The Mount Sinai Acute GVHD International Consortium (Harris et al, Biol Blood Marrow Transplant 2016)*

### *1.4.1.2 Frequency of aGVHD*

Overall, aGVHD affects approximately 55–75 % of children treated with HSCT (34, 35) and grade 2–4 aGVHD approximately 40 % (11, 36). If severity grades 2-4 of aGVHD are considered, the skin is the most frequently affected, followed by the GI tract and the liver (36), with an estimated incidence of aGVHD with involvement of the gastrointestinal tract (GI-aGVHD) in children of approximately 15–25 % (36, 37).

However, in severe aGVHD, the proportion of affected organs might be different (15, 38). In a retrospective study including 50 children with aGVHD grade 3-4, 94 % (47/50) had involvement of the gastrointestinal (GI) tract, either as a solitary manifestation of aGVHD or together with simultaneous aGVHD in the liver or the skin (15).

#### *1.4.1.3 Risk factors for development of aGVHD*

The most important risk factor for development aGVHD is a HLA mismatch between the donor and recipient (13-15). However, several other risk factors have been identified, such as recipient age >12 years at the time of HSCT (39), malignant disease as indication for the transplantation (11), use of an unrelated donor or a female donor to a male recipient (14), use of peripheral blood stem cells as the stem cell source (40), poor oral nutrition (41, 42), previous damage to the GI tract (43-45), and conditioning with total body irradiation (TBI) (46).

### 1.4.2 Chronic GVHD

Symptoms of cGVHD may develop at any time after engraftment. The clinical phenotype of cGVHD mimics autoimmune disease and may affect almost every organ system in the body (47). Presentation of cGVHD may include dry eyes, lichenoid lesions of the oral cavity, scleroderma, alopecia, joint stiffness, hepatitis, exocrine pancreatic insufficiency, wasting syndrome, obstructive lung problems, thrombocytopenia, and lymphocytopenia (28).

The *NIH* has developed diagnostic criteria for cGVHD to improve the diagnostic accuracy and reproducibility of cGVHD in clinical research (28). In clinical practice, however, many patients will not meet the *NIH* criteria but may still be helped by cGVHD treatment (47).

Based on the *NIH* criteria, findings solely of esophagus strictures or webs are accepted diagnostic manifestations of gastrointestinal c-GVHD (GI-cGVHD) (28). Thus, common GI symptoms of GI-aGVHD and GI-cGVHD, such as diarrhea, nausea or vomiting, and anorexia, are, in accordance with the *NIH* criteria, classified as GI-aGVHD (28).

#### *1.4.2.1 Frequency of cGVHD*

Based on the *NIH* cGVHD consensus criteria, approximately 20–40 % of children undergoing HSCT develop cGVHD (48, 49), with the mouth, skin, eyes, and lungs as the most commonly affected organs (48, 49). By contrast, esophageal strictures or webs are rare (48).

#### *1.4.2.2 Risk factors for development of cGVHD*

A previous history of aGVHD is the strongest risk factor for the development of cGVHD (14, 48, 49). Thus, the risk factors associated with the development of aGVHD are also valid risk factors for the development of cGVHD (14).

## 1.5 HUMAN LEUKOCYTE ANTIGENS

HLA are highly polymorphic cell surface immunoglobulins that are encoded on the short arm of chromosome 6. HLA have both antigenic and antigen presenting properties.

Two classical classes of HLA have been defined: HLA 1 and 2. HLA class 1 are expressed on all nucleated cells, whereas HLA class 2 are predominantly expressed on antigen-presenting cells (APCs), such as dendritic cells, macrophages, and bone marrow derived B-lymphocytes

(B-cells). HLA class 1 presents peptides from intracellular pathogens or peptides of intracellular components on the cell surface. The HLA class 1 + ligand complex is recognized by CD8+ T-cells. HLA class 2 presents extracellular components, such as fragments from extracellular bacteria, for the immune system. The activation of CD4+ T-cells depends on recognition of the HLA class 2 + ligand complex.

### 1.5.1 T-cell alloreactivity

T-cell alloreactivity is triggered by genetic differences between two different individuals and is fundamental in the pathophysiology of aGVHD (50). However, alloreactive T-cells are also essential in the desirable GVT reaction (51).

#### *1.5.1.1 HLA-based alloreactivity*

The alloreaction in aGVHD is primarily based on differences in HLA between the donor and recipient. The reaction is induced when donor T-cells recognize the non-self HLA+ligand complex in the recipient. That identification triggers an immune response that causes tissue damage (52).

#### *1.5.1.2 Non-HLA-based alloreactivity*

Non-HLA-based alloreactivity refers to T-cells of donor origin recognizing peptides encoded by polymorphic genes outside the HLA locus as foreign. These immunogenic peptides are called minor histocompatibility antigens (mHA).

Development of aGVHD in an HLA identical HSCT setting has been associated with differences in mHA between the donor and recipient (53). Furthermore, the increased risk of aGVHD associated with female (X X) to male (X Y) transplantation has been associated with mHA encoded on the recipient Y chromosome (54, 55).

#### *1.5.1.3 Alloreactivity in GVT*

GVT alloreaction refers to properties of the graft that eradicate tumor cells in the recipient. Subsets of the graft T-cells, such as natural killer (NK) cells and γδ-T-cells, have been identified as important for this reaction (20).

#### *1.5.1.4 The dual effect of mHA alloreactivity*

The GVT reaction is closely tied to the risk of aGVHD (53). Which immunological phenotype that will predominate have been proposed to depend on whether the mHA that triggers the alloreaction is broadly expressed on many different cell types or whether it is restricted to cells of the hematopoietic system (53). If mHA is restricted to cells of the hematopoietic system, then a GVT reaction will predominate (53). However, if the mHA is broadly expressed on many cell types and tissues, then the probability of occurrence of both GVHD and GVT increases. Broadly expressed mHA has also been proposed to explain the inverse association between the disease severity grade of aGVHD and the risk of relapse (33).

Taken together, the current data indicate that low-grade aGVHD might be a beneficial sign in individuals undergoing HSCT due to malignant blood disease. This is because low-grade aGVHD might indicate an alloreaction, based on mHA, that is predominantly expressed on hematological cells and thus on the malignant cells as well.

## 1.6 PATHOPHYSIOLOGY OF ACUTE GRAFT-VERSUS-HOST DISEASE

In 1966, Rupert E Billingham formulated the following three prerequisites for development of GVHD: the graft must consist of immune cells (T cells), the recipient should not be able to eliminate the transplanted cells, and the graft and the recipient must express different tissue antigens (56). The prerequisites formulated by Billingham have been confirmed in all essential aspects, even though data concerning the pathophysiology of GVHD is primarily based on knowledge derived from animal studies (57).

### 1.6.1 Development of acute GVHD

Development of aGVHD is a multistep process. The basis of the process is a HLA or mHA mismatch between the donor and recipient (Figure 2).

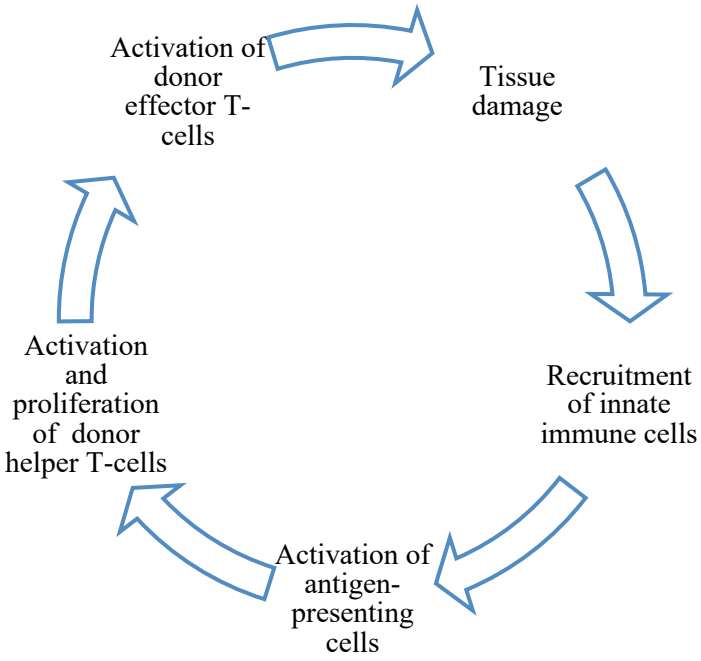


**Figure 2.** The pathophysiology of acute graft-versus-host disease

#### *1.6.1.1 Tissue damage and activation of innate immunity*

The aGVHD process is initiated by tissue damage generated by the conditioning regimen or other tissue-damaging processes (43-45, 58). The tissue damage will cause a release of

intracellular molecules and may also make possible a translocation of extracellular/exogenous molecules.

Tissue damage of the GI tract will enable translocation of luminal exogenous molecules, such as bacteria or bacterial fragments, into the bowel wall. If bacteria are translocated, then blood stream infections may occur prior to clinically apparent aGVHD (59). Furthermore, fragments of bacteria, such as endotoxin/lipopolysaccharides (LPS), may also adhere to toll-like receptors (TLRs) on various cell types in the bowel wall, including epithelial cells, fibroblasts, dendritic cells and macrophages (60). In these cell types, the TLR-LPS complex activates the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, and IL-12 (60). These cytokines will, among other things, act as chemo-attractants, as well as inducing increased expression of adhesion molecules on endothelial cells. These responses, all together, lead to the migration of neutrophils, eosinophils, and macrophages into the damaged tissue (52, 61). However, some cytokines, such as TNF-α, may cause further tissue damage by induction of programmed cell death, i.e., apoptosis (62).

Injured or necrotic cells release several different molecules, such as uric acid, adenosine triphosphate (ATP), and IL-33. These molecules, together with LPS, IL-12, and TNF-α, have properties that activate APCs (63). Activated APCs may in the following step activate T-cells of donor origin (63), thereby bridging tissue damage and innate immune activation with an adaptive immune response during the development of aGVHD.

#### *1.6.1.2 Activation of T-cells of donor origin*

Activated CD8+ cytotoxic effector T lymphocytes (CTLs) are the major effector cells in the pathophysiology of aGVHD. Induction of lysis, due to perforation of the target cell membrane or induction of apoptosis, are the mechanisms by which CTLs induce tissue damage in aGVHD (64).

The activation of CTLs may be triggered: *i*) by HLA class 2 on APCs, presenting degraded, disparate HLA or mHA proteins to Helper CD4+ T-cells (Th cells) of donor origin, or *ii)* directly by HLA class 1 molecules (52). The HLA class 2 pathway induces proliferation and differentiation of the Th-cells, which will be followed by production of certain cytokines, such as IL-2 and interferon-gamma (IFN-γ) (52, 65). These cytokines are, among other things, responsible for the activation of CTLs as well as macrophages (52).

Taken together, results from previous studies show that activated CTLs are the major effector cells in the pathophysiology aGVHD. However, other cell types, such as monocytes/macrophages, in concert with the toxic effect exerted by different cytokines, also contribute to the aGVHD-related tissue destruction (52, 66).

#### *1.6.1.3 Commensal microbiota*

The impact of the GI microbiota on the pathophysiology of aGVHD was discovered by *Van Bekkum et al.* in 1974 (67). These researchers observed that the incidence of and the mortality

due to aGVHD were significantly higher in conventional mice than in mice kept in germ-free state after lethal irradiation followed by allogeneic bone marrow transplantation (67).

The concept discovered by *Van Bekuum et al* has been difficult to apply to human HSCT recipients. Only approximately 50 % of children treated from day -10 until day +30 with high doses of non-absorbable antimicrobial drugs and nursed in strict isolation were able to achieve "germ-free" GI tracts (68). Instead, treatment with a broad-spectrum antibiotic at the time point of the HSCT has in human HSCT recipients been associated with a reduced diversity of the gut commensal microbiota (dysbiosis) (69), increased risk of GI-GVHD (69), and an increased probability of mortality within 3 years post HSCT (70, 71).

Changes in the composition of the microbiota in HSCT patients include increased proportions of *Enterococcus* spp. (69, 72) and *Escherichia coli* (*E. coli*) (71, 72), as well as reduced proportions of the *Clostridium* cluster (69, 73). These changes in the microbiota may, however, not be fully explained by antibiotic treatment (70). Another contributing factors may be a decreased number of Paneth cells in the GI tract epithelium of the small intestine, a finding that has been correlated with GI-GVHD (74). Paneth cells secrete anti-bacterial peptides, such as regenerating islet-derived 3 alpha (Reg-3-α), that affect *E. coli* without affecting the *Clostridium* cluster or other commensal bacteria (75). Thus, loss of Paneth cells increases the survival possibilities for *E. coli*, while decreasing the survival properties for the *Clostridium* cluster.

The *Clostridium* cluster produces short fatty acid chains, such as butyrate, an important nutrient for the intestinal epithelial cells (76). Therefore, a reduction in the number of clostridia may induce a nutritional deficiency in the GI tract mucosa. This deficiency may cause GI tract damage, thereby increasing the risk for development of aGVHD (73, 76).

#### *1.6.1.4 Suppression of the immune response*

A non-regulated immune response may cause a vicious cycle, resulting in deleterious tissue damage to the host (Figure 2). Avoidance of this cycle requires suppression of the immune response. However, in the context of HSCT, this suppression should preferably not encroach on the GVT reaction.

Infusion of regulatory T-cells (T-reg, expressing CD4+, CD25+, and Forkhead Box Protein 3 [FoxP3]) at day -4 has been associated with a reduced risk for the development of aGVHD without impacting the GVT reaction (77).

However, in a clinical situation with ongoing aGVHD, expression of mRNA for transcription factor Foxp3 in peripheral blood mononuclear cells was inversely related to the clinical severity grade of aGVHD (78)**.** The highest levels of Foxp3-mRNA expression were observed in patients without aGVHD (78). Furthermore, a biopsy-based study that included patients with histopathology-based GI-GVHD diagnosis and healthy controls revealed similar T-reg/CD8+ T-cell ratios in the healthy controls and in those with GI-GVHD (79). That finding was interpreted as an insufficient upregulation of T-regs to counteract aGVHD (79).

Taken together, the data indicate that a numerical and/or functional reduction of T-regs may cause insufficient suppression of the immune response in aGVHD.

## 1.7 TREATMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE

### 1.7.1 First-line treatment

The first-line treatment of grade 2–4 aGVHD is 2 mg/kg bodyweight/day of methylprednisolone or an equivalent glucocorticoid for seven days, followed by slow tapering according to the response (9, 80). Despite a sparse body of evidence (81), mainly non-absorbable corticosteroids, like beclomethasone or budesonide, are often added to methylprednisolone in patients with GI-GVHD (9).

Upper GI tract manifestations of aGVHD have been shown to be more responsive to treatment with steroids (82, 83). Therefore, in individuals solely affected by upper GI tract aGVHD, a proposed treatment regimen is an initial ten-day course with methylprednisolone at 1 mg/kg bodyweight /day, followed by a fifty-day course of beclomethasone or budesonide (9).

Overall, the probability of a treatment response to glucocorticoids is lower with a higher disease severity grade of aGVHD (80). Non-methylprednisolone-responsive aGVHD i.e., steroid refractory aGVHD (SR-aGVHD), has been defined as one of the following: *i*) progression of aGVHD manifestations despite treatment with ≥2 mg/kg bodyweight/day of methylprednisolone for 3–5 consecutive days; *ii*) failure to improve within 5–7 days of treatment initiation, or; *iii*) incomplete response after more than 28 days of steroid treatment (32).

The incidence of overall SR-aGVHD in children has been estimated to be 35 % (84). The corresponding incidence in lower GI tract SR-aGVHD has been reported as 30–52 % (36, 84).

### 1.7.2 Second-line treatments

Several agents, such as mycophenolate mofetil or mesenchymal stromal cells, as well as monoclonal antibodies directed against the IL-2 receptor on T-cells, against TNF-α, or against endothelial cell adhesion molecules, have been evaluated or are currently under evaluation, as second-line treatments, either alone or in combination with methylprednisolone. However, none of them have yet shown convincing long-term efficacy (80).

A novel second line aGVHD treatment strategy is inhibitors of Janus-activated kinase 1 and 2 (JAK 1/2). The JAK 1/2 inhibitors target signaling events downstream of cytokine receptors (80). One JAK 1/2 inhibitor, ruxolitinib, has shown promising preliminary results in SR-aGVHD but has also been associated with serious side effects, such as neutropenia and infections (85). Thus, further studies are needed to evaluate the clinical usefulness of ruxolitinib.

## 1.8 SURVIVAL

Currently, approximately three out of four children survive three years post-HSCT, when all underlying diagnoses of HSCT are considered (11, 34).

Several factors that pre-exist before HSCT have been associated with a less favorable probability of survival. These factors include; malignant blood disease as indication for the transplantation; HSCT performed in children below one year of age; HLA mismatch between donor and recipient; use of an unrelated donor; a serological-based CMV mismatch between the donor and recipient; female-to-male transplantation; and donor age > 33 years (11, 13, 18, 86, 87). Factors added after the transplantation that have a substantial negative impact on survival are relapse of the underlying diagnosis, infections, and aGVHD grade 3–4 (18, 33, 34).

The probability of three-year survival in a cohort of children with aGVHD following HSCT due to leukemia were 79–76 % for grade 0–2, 67 % for grade 3, and 42.5 % for grade 4 aGVHD (33). The corresponding figure for two-year overall survival in SR-aGVHD has been estimated to be 35 % (84).

# 2 THESIS SPECIFIC BACKGROUND

## 2.1 RATIONALE FOR THE THESIS

Gastrointestinal aGVHD is associated with bothersome symptoms and poor outcome. The treatment of choice, glucocorticoids, is accompanied with risks, such as viral infections. In the absence of a response to glucocorticoid treatment, the overall expected two-year survival is reduced by approximately 50 %. Thus, a reduction in the risk of development of GI-aGVHD is important.

Histopathological confirmation of symptom-based GI-aGVHD is recommended (7-9). However, the clinical importance of this recommendation applied to children is hampered by *i*) the lack of robust data supporting an influence of endoscopy-guided histopathological assessment on treatment decisions, *ii*) the lack of consensus regarding the minimal histological criteria for the GI-GVHD diagnosis and, *iii*) the absence of clarity regarding the optimal extent of the endoscopic procedure when the indication of the procedure is to confirm symptom-based GI-aGVHD. Thus, several clinical aspects of diagnostic of pediatric GI-GVHD, based on endoscopy-guided histopathological assessment, require deeper knowledge.

## 2.2 DEFINITIONS

In accordance to the *NIH,* the minimal histopathological criterion necessary to diagnose gastrointestinal GVHD, is finding of crypt apoptosis (8). Apoptosis is, furthermore, a sign of ongoing tissue damage and has therefore been proposed to be termed "active" GVHD, regardless of the traditional distinction between acute, chronic, and overlap GVHD (88). Henceforth in this thesis, the symptom-based diagnosis of gastrointestinal GVHD has been termed *GI-aGVHD.* Furthermore, *GI-GVHD* has been used for histopathology-based "active" gastrointestinal GVHD.

## 2.3 DIFFERENTIAL DIAGNOSIS

Infections in the GI tract or side effects of drugs, including the conditioning regimen, may cause symptoms indistinguishable from those of GI-aGVHD. In children with diarrhea post-HSCT, an infectious cause has been found in 10–20 %, with *Clostridium difficile (C. difficile)* (89), rota-, noro-, adenovirus, and CMV as the most common agents (90-93). However, diarrhea caused by infectious parasites or bacterial agents other than *C. difficile*, has been reported to be rare (94).

### 2.3.1 *Clostridium difficile*

*C. difficile* infections (CDIs) have been observed in approximately 8–9 % of children undergoing HSCT (37, 89). CDI has a recurrence rate of approximately 20 % (89). The diagnosis of CDI rests on a positive fecal toxin assay, together with typical clinical symptoms and exclusion of other causes of diarrhea (95). Severe CDI, in the form of pseudomembranous colitis, is accompanied by typical endoscopic and histological findings (96). However, in immunosuppressed patients, the macroscopic appearance of pseudomembranous colitis might not be readily evident (97).

### 2.3.2 Cytomegalovirus

Within one-year post-HSCT, the cumulative incidence of CMV disease has been estimated as approximately 5–6 %, with pneumonia, gastroenteritis, hepatitis, and retinitis as the most frequently observed manifestations (93, 98).

The pre-HSCT serological CMV status of the donor and the recipient influences the risk of CMV disease, with the highest risk observed in a CMV seronegative donor to a CMV seropositive recipient (93, 99). Two different strategies have been developed to prevent the onset of CMV disease: prophylactic administration of anti-CMV drugs and a pre-emptive strategy. The latter includes CMV polymerase chain reaction (PCR)-based monitoring, once or twice weekly, and early treatment if CMV viremia is detected (100).

Symptoms of CMV disease in the gastrointestinal tract include weight loss, dysphagia or odynophagia, nausea and vomiting, abdominal pain, diarrhea, and fever. Any of these symptoms, together CMV immunohistochemical staining, can serve as the basis for the diagnosis of CMV gastroenteritis (101).

### 2.3.3 Post-transplant lymphoproliferative disease

Post-transplant lymphoproliferative disease (PTLD) is a potentially life-threatening condition (102) that affects less than 2 % of HSCT patients (103). In pediatric HSCT patients, > 90 % of PTLD cases are associated with EBV and are of B-cell origin (104). EBV-induced transformation of B-cells occurs most frequently in a setting of impaired cellular immunity arising from ongoing immunosuppressive prophylaxis or treatment (104). Other risk factors for development of PTLD post-HSCT include HLA mismatch between the donor and recipient, RIC, a serological-based EBV-positive donor and an EBV-negative recipient, aGVHD grade 2–4, and splenectomy performed pre-HSCT (102). In order to limit the risk of PTLD development post HSCT, PCR-based EBV-DNA viral load monitoring, at least once weekly for at least three months, is recommended (103). If EBV viremia is detected, preemptive treatment should be considered (103).

The clinical presentation of PTLD most frequently includes lymphadenopathy, fever, and weight loss. Abdominal problems such as hepatosplenomegaly and gastrointestinal symptoms like diarrhea and hematochezia, have been observed in approximately every fifth patient with PTLD, based on a mixed-age HSCT population (105).

The final diagnosis of gastrointestinal PTLD relies on histopathology, including morphological assessment, immunophenotyping, and EBV-encoded RNA (EBER) in situ hybridization (106). Treatment options include reduction of immunosuppression, rituximab (anti-CD20 monoclonal antibodies), or chemotherapy (104).

### 2.3.4 Typhlitis

Typhlitis, or neutropenic enterocolitis, is a rare but life-threatening consequence of severe mucosal damage of the GI tract due to chemotherapy. Involvement of the cecum and right colon is seen in almost all affected individuals. Bowel wall perforation and sepsis may occur and require surgical resection and antibiotics (107).

Taken together, the present evidence indicates that diarrhea during the post-HSCT period may have multiple causes. Infection of the GI tract needs to be considered early in the diagnostic procedure of individuals with clinically suspected GI-aGVHD, preferably before endoscopy is performed.

## 2.4 RISK FACTORS FOR DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE IN THE GASTROINTESTINAL TRACT

Risk factors for the development of GI-aGVHD have not been studied in depth. However, addition of total body irradiation to the conditioning regimen (46), infection with enteric viruses, such as adeno- and norovirus, at the time of HSCT (108), and CDI (109) have been associated with an increased risk of subsequent development of GI-aGVHD. A common feature of these factors is that they have the potential to cause damage to the GI tract, thereby acting as initiators of the pathophysiological process of GI-aGVHD (Figure 2).

Parenteral nutrition as the only feeding route (110) and administration of broad-spectrum antibiotics, such as ciprofloxacin, at the time of the transplantation (69) have also been associated with an increased risk GI-aGVHD. The mechanism of action that explains the association between antibiotics and GI-aGVHD has not been clarified. However, total parenteral nutrition as a risk factor for GI-aGVHD might be explained by an induction of tissue damage, including impaired barrier function and apoptosis, if the GI tract is not exposed to food (111, 112).

### 2.4.1 Busulfan and cyclophosphamide conditioning, with or without the addition of melphalan

Busulfan (Bu) and cyclophosphamide (Cy) conditioning was developed in 1983 by *Santos et al*. from the Johns Hopkins group as a way to avoid irradiation-induced conditioning-related toxicity (19). A third alkylating agent, melphalan (Mel), is sometimes added to the BuCy conditioning to further reduce the risk of graft failure and relapse (113).

In the original BuCy regimen used by the Johns Hopkins group, Bu was administered orally. However, the pharmacokinetics of Bu is unpredictable and shows substantial interindividual

variation in bioavailability, particularly in children (114). Therefore, an increased risk of drug toxicity has been observed in individuals with high absorption of Bu and an increased risk of relapse and graft failure in those with low absorption (115). Management of the unpredictable pharmacokinetics requires either intravenous administration of Bu or therapeutic drug monitoring followed by dose adjustments in patients treated with oral administration (19).

The main toxic manifestations of BuCy conditioning include sinusoidal obstructive syndrome (116), hemorrhagic cystitis (117), and drug toxicity affecting the gastrointestinal tract, the kidneys, and the central nervous system (118). Furthermore, the addition of Mel to BuCy has been found to increase the risk of aGVHD (119). However, whether the addition of Mel to the BuCy conditioning increases the risk of GI-aGVHD is not known.

## 2.5 DIAGNOSTIC OF ACUTE GRAFT-VERSUS-HOST DISEASE IN THE GASTROINTESTINAL TRACT

Acute GI-GVHD is primarily a symptom-based diagnosis. However, symptoms due to GI-aGVHD are nonspecific. Additional diagnostic tools to support or confirm the GI-aGVHD diagnosis are therefore of clinical importance. The *MAGIC* consensus GVHD research guidelines state that histopathological confirmation of GI-GVHD is the most important available diagnostic test for attributing gastrointestinal symptoms to GI-aGVHD (7) . However, in children (below 18 years of age), in contrast to adults, esophagogastroduodenoscopy (EGD) and colonoscopy, with or without ileal intubation, should be performed under general anesthesia, either in an operating theater or endoscopic procedure room (120, 121). Thus, endoscopy in children is a logistically complex and time-consuming procedure that may interfere with prompt management (122). Consequently, the potential clinical value of diagnostic tools other than histopathology might be of particular importance in children.

### 2.5.1 Imaging

Findings on computed tomography (CT) (123), magnetic resonance imaging (MRI) (124), and transabdominal ultrasonography (US) (125) in individuals with histopathologically proven GI-GVHD have been associated with non-specific findings, such as bowel wall thickening and abnormal mucosal enhancement. Thus, CT, MRI, and US add nonspecific information of less clinical importance to support the GI-aGVHD diagnosis.

### 2.5.2 Plasma and fecal biomarkers

The antimicrobial peptide, Reg-3-α, produced by the GI tract epithelium has been evaluated as a biomarker of GI-GVHD (126). Significantly higher plasma levels of Reg-3-α were observed in individuals with histopathology-based lower GI tract GVHD versus individuals without GVHD or solely with skin GVHD. The highest level of Reg-3-α was found in individuals with severe GI-GVHD (126). However, confirmatory studies are needed before Reg-3-α can be incorporated into standard clinical practice (80).

Calprotectin is a protein that is mainly released to the bowel lumen from neutrophils during GI tract inflammation. Analysis of fecal calprotectin is a routine test in the context of inflammatory bowel disease (IBD); during the primary diagnostic workup, to evaluate IBD treatment response and, to identify IBD flare-up. Elevated fecal calprotectin levels have also been found in GI-aGVHD (127, 128). Furthermore, a positive correlation has been observed between levels of fecal calprotectin and disease severity grade of GI-aGVHD (127), However, the ability of fecal calprotectin to differentiate between GI-aGVHD and non-GI-aGVHD causes of GI tract symptoms has not been studied in depth (129). In summary, fecal calprotectin shows promising results as a diagnostic tool for GI-aGVHD, but its specificity needs further clarification.

### 2.5.3 Macroscopic appearance of the GI tract mucosa

In endoscopies performed to confirm symptom-based GI-aGVHD, histopathology reveals GI-GVHD in approximately every second endoscopic occasion with a normal macroscopic appearance of the GI tract mucosa (130, 131). Therefore, endoscopy, solely based on visual inspection of the GI tract mucosa, has low additive value in the diagnostics of GI-aGVHD.

## 2.6 HISTOPATHOLOGY-BASED DIAGNOSIS OF ACUTE GRAFT-VERSUS-HOST DISEASE IN THE GASTROINTESTINAL TRACT

Abnormal histology related to GI-GVHD is primarily located in the crypts of the GI tract epithelium, which are in an area with a high proportion of intestinal stem cells and Paneth cells.

The histological hallmark of GI-GVHD is crypt apoptosis (8, 132). Other histological findings include crypt destruction, mucosal denudation, reduced numbers of Paneth cells in the small intestine, and sparse infiltration of lymphocytes and eosinophils in the lamina propria (74, 133).

### 2.6.1 Minimal criterion of histopathology-based GI-aGVHD

The minimal histopathological criterion of GI-GVHD has not been established (8). However, the *NIH* (2005/2014) defines the histopathological threshold of GI-GVHD as detection of at least one apoptotic body in a crypt per biopsy piece (8, 88).

Nevertheless, apoptosis is not a unique histological finding of GI-GVHD, and particularly not findings of less than one apoptotic body per biopsy piece (134, 135). Therefore, to increase the diagnostic specificity, more than one apoptotic body per biopsy piece, have been proposed as a minimal criterion (136-138). However, due to the overall trade-off between sensitivity and specificity, increased number of apoptotic events needed for the GI-GVHD diagnosis may decrease the diagnostic sensitivity, thereby potentially increasing the risk that clinically relevant cases of GI-GVHD will remain undetected.

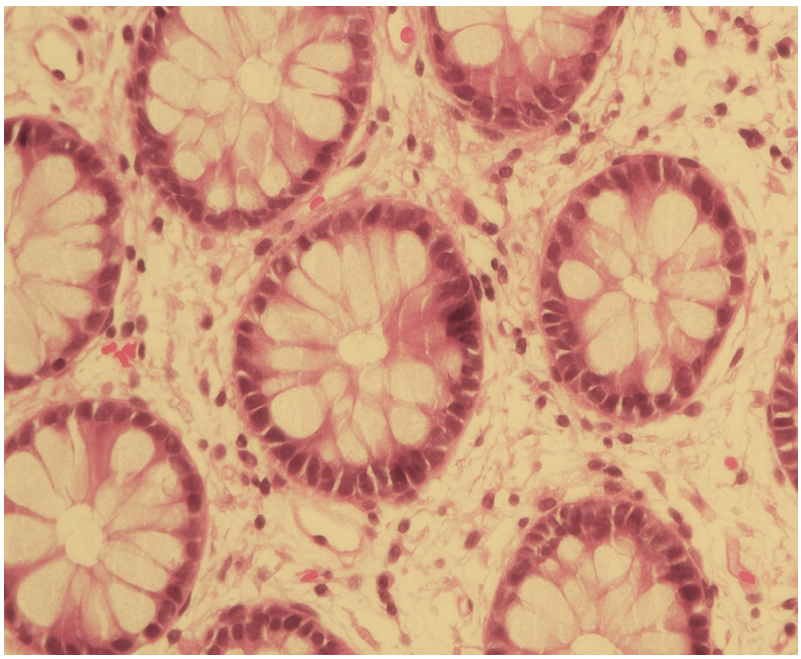

**Figure 3:** Normal colonic mucosal histology

### 2.6.2 Histopathology-based disease severity grading

Histopathology-based disease severity scoring of GI-GVHD is most often based on the four-tiered scoring system described by *Lerner et al.* (139);

- Grade 1: Apoptotic epithelial cells, without crypt loss (Figure 4)
- Grade 2: Apoptotic epithelial cells and individual crypt loss or crypt destruction (Figure 5)
- Grade 3: Apoptotic epithelial cells and *i*) contiguous areas of multiple crypt loss or crypt destructions, and/or, *ii*) focal mucosa denudation, and/or *iii*) focal ulceration
- Grade 4: Diffuse denudation of the epithelium or ulceration. Apoptotic epithelial cells, not always possible to detect (Figure 6)

Grading of the disease severity of GI-GVHD based on histopathology is somewhat controversial. However, the histopathology-based disease severity may add prognostic information of clinical value. For example, Lerner grades 3–4 versus grade 1-2, have been associated with an increased risk of SR-GI-aGVHD (140) and reduced probability of survival (135).

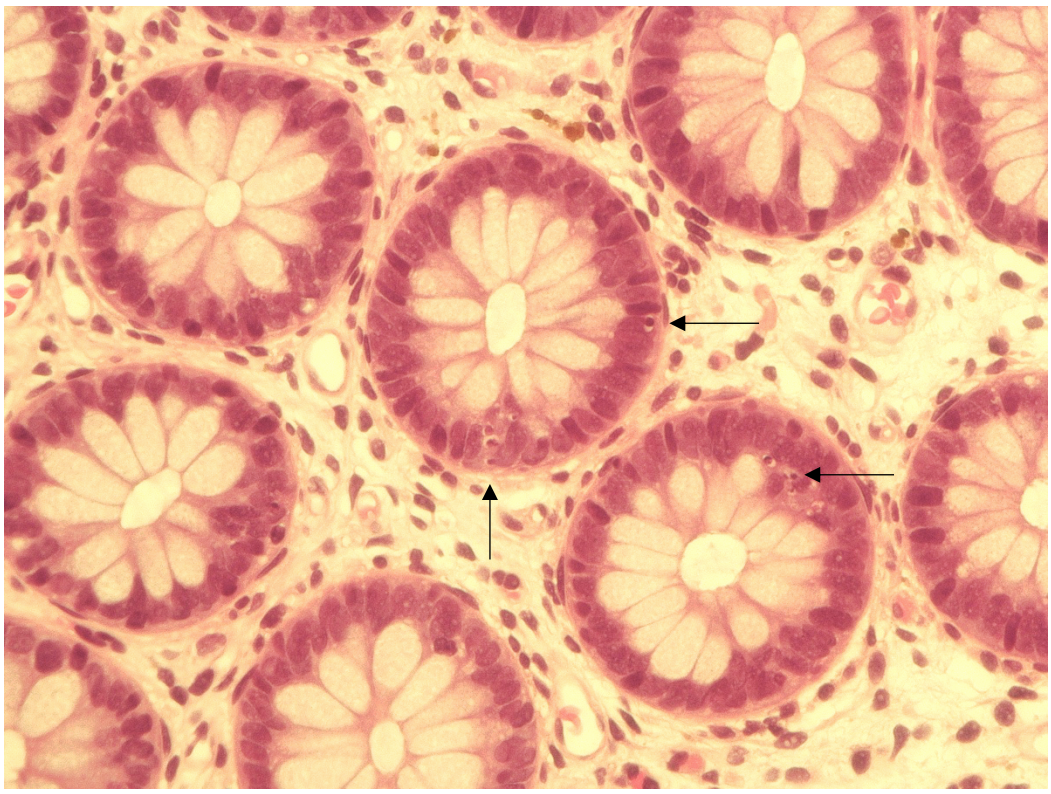

**Figure 4**. Apoptotic bodies in colon, indicated with arrows (Lerner grade 1)

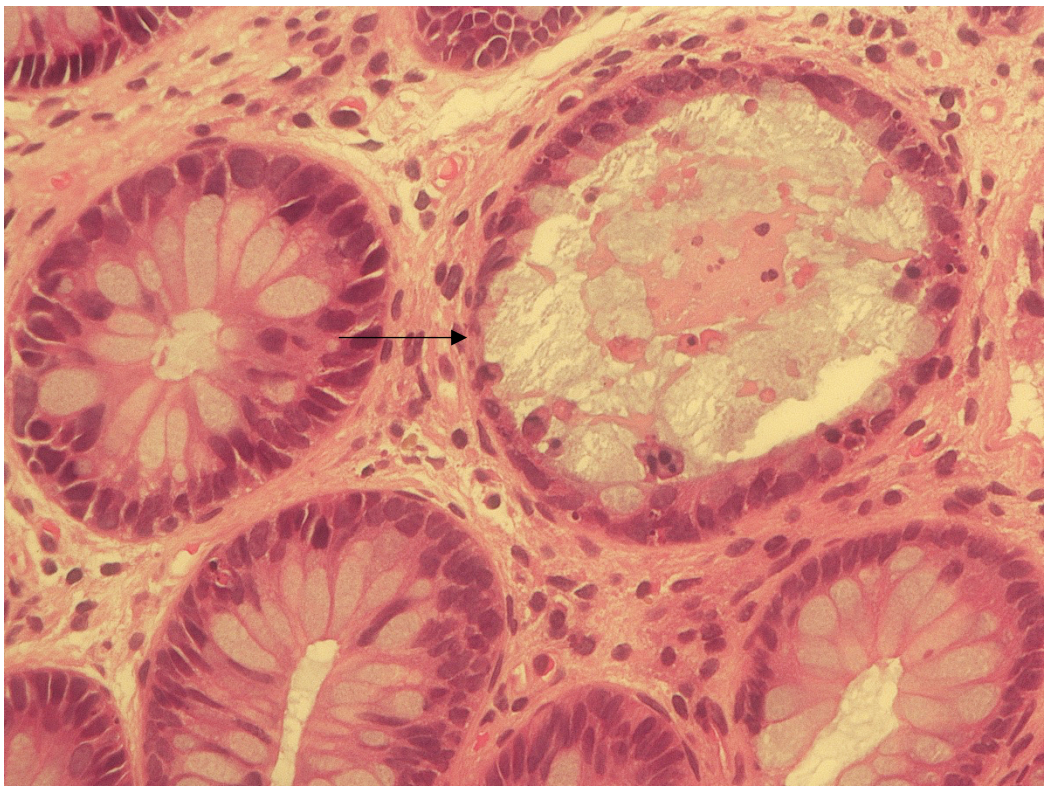

**Figure 5.** Lerner grade 2 with apoptotic epithelial cells and a single crypt destruction

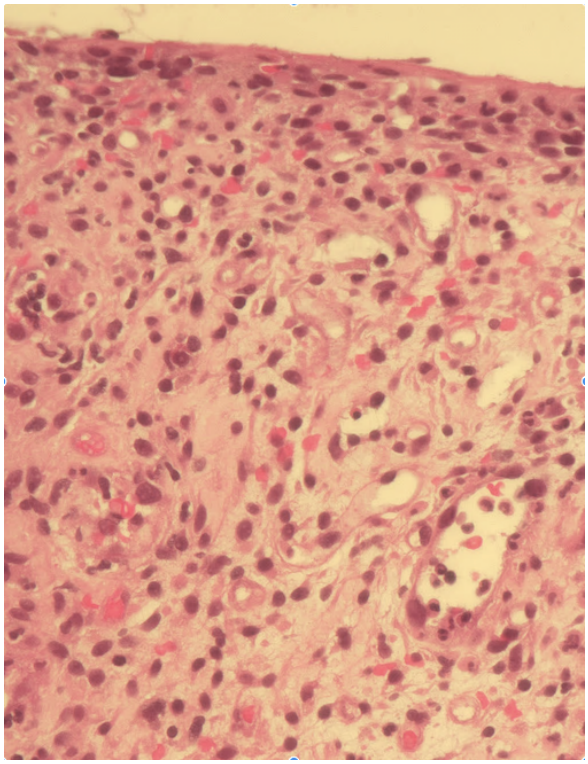

**Figure 6.** Lerner grade 4 with complete destruction of the colonic mucosa including denudation of the epithelium

### 2.6.3 False negative interpretation of histopathology-based GI-GVHD

GI-GVHD may remain undetected during the histopathological assessment due to the following (8, 133):

- Small biopsy pieces, insufficient to detect infrequent apoptotic bodies
- Biopsy sampling performed within an ulcer, rather than in the transitional area to intact mucosa
- Ongoing anti-GI-GVHD treatment at the time of biopsy sampling
- Suboptimal processed and stained biopsy slides
- Serial sections that do not go down to the base of the crypts
- Suboptimal extent of the endoscopic procedure
- Interobserver disagreement between different pathologists

#### *2.6.3.1 Choice of endoscopic procedure*

Sigmoidoscopy, in contrast to EGD and colonoscopy/ileocolonoscopy, represents an endoscopic procedure that might be performed under minimal sedation in children (141-143). Thus, evaluation of the diagnostic reliability of the GI-GVHD diagnosis based on biopsies from the rectosigmoid area is important.

Four previous pediatric studies (130, 144-146) have evaluated sigmoidoscopy as a potential first choice endoscopic procedure in children with symptom-based GI-aGVHD. Two of four studies were based on biopsy sampling from full upper and lower endoscopy (144, 145). In these studies, nine of 13 (69 %) and 13 of 18 (72 %) endoscopic occasions with histopathology-based GI-GVHD anywhere in GI tract were simultaneously detectable in biopsies from the rectosigmoid area (144, 145).

In the remaining two studies, the sensitivity of the histopathology-based GI-GVHD diagnosis was compared, based on biopsy sampling from the rectosigmoid area versus the upper GI tract (130, 146). In one of these studies, all (n=9) children with a histopathology-confirmed GI-GVHD diagnosis in biopsies from the upper endoscopic procedure were confirmed in biopsies from the rectosigmoid area (146). In the other study (n=22), the sensitivity for the GI-GVHD diagnosis based on biopsies from the rectosigmoid area was 77 % (130).

Taken together, it remains unclear, due to limited sample sizes in previous pediatric studies, if sigmoidoscopy can be recommended as preferred first choice of endoscopic procedure in children with clinically suspected GI-aGVHD (130, 144-146).

#### *2.6.3.2 Interobserver disagreement*

In a histopathology-based study performed by five pathologists using 33 biopsies with GI-GVHD, full agreement of the GI-GVHD diagnosis among the pathologists was observed in 23 biopsies (70 %) (132). The corresponding figure of agreement for grading of GI-GVHD in accordance to *Lerner et al.* was 48 %. In another adult patient study that included 217 endoscopic occasions performed due to clinically suspected GI-GVHD, the clinical standard histopathological assessment (CSHA) confirmed the GI-aGVHD diagnosis in 166 (53 %) endoscopic occasions (134). In a subsequent retrospective, independent, histopathological assessment (RIHA) based on the *NIH 2014* criteria, the corresponding result was 173/217 (80 %) (134). None of these previously mentioned studies included a detailed analysis of the potential clinical consequences related to the differences in the assessments (132, 134).

### 2.6.4 False positive interpretation of histopathology-based GI-GVHD

False positive interpretation of histopathology-based GI-GVHD may reflect apoptosis-inducing factors other than GI-GVHD (Table 3), as well as interobserver disagreement.

**Table 3**. Potential apoptosis inducing factors, that may mimic histopathology-based gastrointestinal GVHD diagnosis

| Histopathological mimic | Main "target" region of the GI tract | Experimental model | References |
|---|---|---|---|
| **CMV** | Entire GI tract | *Human* | *Karamchandani 2018 (147)* |
| ***Salmonella, Shigella, Yersinia, Campylobacter spp.*** | Rectum to terminal ileum | *Human and mice* | *Navarre 2000 (148), Bucker 2018 (149)* |
| **Norovirus** | Terminal ileum to duodenum | *Human* | *Troeger 2009 (150)* |
| **Rotavirus** | Terminal ileum to duodenum | *Human* | *Chaibi 2005 (151)* |
| **Adenovirus** | Entire GI-tract | *Human* | *Westerhoff 2017 (107)* |
| ***Clostridium difficile*** | Rectum to caecum | *Mice* | *Chumbler 2016 (152)* |
| ***Helicobacter pylori*** | Gastric antrum and corpus | *Human* | *Moss 2001 (153)* |
| **Conditioning regimen** | Entire GI tract | *Human* | *Epstein 1980 (154), Jalili-Firoozinezhad 2018 (155)* |
| **Mycophenolate mofetil** | Entire GI tract | *Human* | *Nguyen 2009 (156), Papdimitriou 2003 (157)* |
| **Tacrolimus** | Unknown | *Mice* | *Yoshino 2010 (158), Fujino 2007 (159)* |
| **Total Parenteral Nutrition/no enteral nutrition** | Unknown | *Mice* | *Cao 2000 (160), Wildhaber2002 (112)* |
| **Non-steroidal anti-inflammatory drugs** | Entire GI tract | *Human* | *Parfitt 2007 (161)* |
| **Bowel preparation** | Rectum to caecum | *Human* | *Westerhoff 2017 (107)* |
| **Proton pump inhibitor** | Gastric antrum | *Human* | *Welch 2006 (162)* |

### 2.6.5 Evaluation of diagnostic tools in the absence of diagnostic reference standard

A reference standard is required for assessment of whether the *NIH 2014* criteria or other thresholds for the GI-aGVHD diagnosis add information that increases or decreases the uncertainty of the GI-aGVHD diagnosis. However, no such reference standard has been established for histopathology-based GI-GVHD (8). Without a diagnostic reference standard, the clinical evaluation of a diagnostic test needs to be based on other markers that can be used to evaluate the post-test versus pre-test probability of a correct diagnosis (163). These markers may include primary disease endpoints, such as therapeutic decisions, or other aspects of patient management or clinical outcome, such as survival (163).

### 2.6.6 Potential clinical consequences of over- and under-diagnosis of GI-GVHD

An association between non-detected histopathology-based GI-GVHD diagnosis and an increased risk of mortality is not yet known. Similarly, whether undetected GI-GVHD increases the risk of potentially avoidable re-endoscopies remains to be established. The latter might of greater clinical relevance in children than adults since *i*) an increasing number of publications have reported that repeated exposure to general anesthesia in children, is harmful (164, 165), at least in children below the age of three years (166); *ii*) biopsy sampling–induced intraduodenal hematoma has been reported to occur more frequently in children with endoscopy performed to confirm clinically suspected GI-GVHD compared to other medical indications (167, 168).

Conversely, overdiagnosis of GI-GVHD may increase the risk of unnecessary treatment with immunosuppressive drugs, thereby increasing the risk of potential avoidable side effects, such as viral infections (169), infectious related death (170), and other drug-associated side effects (171).

### 2.6.7 Reporting final histopathological diagnosis in accordance with the NIH

To facilitate a comparison of the outcome between different studies within the field of histopathology-based GI-GVHD, the *NIH* recommends standardized reporting of the final histopathological diagnosis, based on the following categories: *not* GVHD, *possible* GVHD, or *likely* GVHD (8). The category *likely* GVHD represents a merged group, previously described as *consistent* and *definite* GVHD (88).

In *possible* GI-GVHD, evidence of GVHD has been found but other explanations of the histopathology picture are possible, such as CMV enteritis or drug-induced mucosal reaction (Table 3). *Likely* GI-GVHD includes clear histopathology-based evidence of GI-GVHD, ranging from minimal to marked GI tract injury (8).

Based on a study including adult HSCT patients with endoscopy-guided histopathological assessment performed due to clinically suspected GI-GVHD, the three-year overall survival was 64 % in individuals classified as *possible* GI-GVHD and 31 % in those classified as *likely* GI-GVHD (135). The overall survival in individuals classified as *possible* GI-GVHD, did not differ compared with those without histopathology-based GI-GVHD. Based on these findings, the authors of the study proposed to merge those with histopathology-based possible GI-GVHD and those without signs of GI-GVHD into one group (135).

### 2.6.8 Final diagnosis in clinical practice

In daily clinical practice, the final GI-aGVHD diagnosis represents an aggregated interpretation of different clinical data, such as:

- Symptoms
- Occurrence of risk factors for the development of GI-aGVHD

- Histopathological findings
- Occurrence of infections or treatment with drugs that may cause GI-aGVHD-like symptoms or may induce GI tract apoptosis
- Simultaneous manifestations of aGVHD in organs other than the GI tract
- Response to anti-GI-GVHD treatment, if that has been started before the final diagnosis has been established

The attributed value of endoscopy-guided histopathological assessment in daily clinical management of adult patients with suspicion of GI-aGVHD was highlighted in a survey of current clinical practice in diagnosis and treatment of aGVHD (172). In that study, 30 of 34 (88 %) European HSCT centers reported that they would perform endoscopy with biopsy sampling before starting anti-GI-GVHD treatment in a patient presenting with diarrhea and decreased oral intake after HSCT (172). Corresponding pediatric studies are lacking.

## 2.7 TREATMENT CHANGES BASED ON HISTOPATHOLOGY FINDINGS

One previous study has evaluated the frequency of treatment changes prompted by results from histopathological assessment, in children with symptom-based GI-aGVHD (173). In that study, 40 % of the participants were on anti-GI-GVHD treatment at the time of the endoscopy. Despite that, only initiation and dose escalating of anti-GI-GVHD drugs were included in the study-specific definition of treatment changes. Thus, neither a dose reduction or withdrawal of anti GI-GVHD drugs nor treatment modifications due to finding an alternative diagnosis were classified as treatment changes.

In summary, no previous pediatric study has evaluated the overall frequency of treatment changes based on endoscopy-guided histopathological assessment performed to confirm symptom-based GI-aGVHD.

# 3 OBJECTIVES AND AIMS

## 3.1 OVERALL OBJECTIVES

- To study the influence of two different conditioning regimens on the incidence of GI-aGVHD in HSCT-treated children
- To evaluate clinical aspects of the currently recommended diagnostic approach to GI-aGVHD, i.e., endoscopy-guided histopathological assessment, applied to pediatric HSCT patients

## 3.2 SPECIFIC AIMS

- To evaluate if addition of Mel to the BuCy conditioning regimen increases the incidence of GI-aGVHD within 100 days post-HSCT in children suffering from juvenile myelomonocytic leukemia (JMML) or myelodysplastic syndrome (MDS) (*paper I*)

- To evaluate the frequency of treatment changes: initiation, dose escalation, tapering, or withdrawal of anti-GI-GVHD drugs and other changes to medication based on endoscopy-guided histopathological assessment performed to confirm symptom-based GI-aGVHD in children (*paper II*)

- To evaluate the frequency of histopathological disagreement of the GI-GVHD diagnosis between CSHA and *NIH* 2014-based RIHA performed blinded by one pathologist (*paper III*)

- To assess the risk of subsequent re-endoscopy within one-year post-HSCT and of death within two years post-HSCT as a potential consequence of mismatch of the GI-GVHD diagnosis between CSHA and RIHA (*paper III*)

- To evaluate if biopsies from the rectosigmoid area versus from the rest of the colon/ileocolon, with or without biopsies from simultaneous upper endoscopy, are equally reliable for establishing a histopathology-based GI-GVHD diagnosis (*paper IV*)

# 4 PATIENTS AND METHODS

## 4.1 PATIENTS

### 4.1.1 Patients: paper I

This was a single center study carried out in children <18 years of age at Karolinska University Hospital Huddinge. The study enrolled all children with HSCT performed during 2000–2010 with the underlying diagnoses of JMML and MDS and with a conditioning regimen of BuCy, with or without addition of Mel. Children with JMML and MDS were chosen since these diagnoses proceed directly to HSCT when the diagnosis has been established (174, 175). Thus, the patients were minimally exposed to chemotherapy prior to HSCT, thereby avoiding GI toxicity that may have influenced the outcome.

A total of 26 patients were identified. However, one child with JMML died prior to neutrophil engraftment, thus, before fulfilling the prerequisite for development of aGVHD, and was therefore excluded.

### 4.1.2 Patients: paper II-IV

*Paper II-IV* enrolled patients from all pediatric HSCT centers in Sweden (Gothenburg, Lund, Stockholm, Uppsala). The participants were identified via hospital record databases, local registries of HSCT-treated children, and databases for pathology.

A primary study population was collected that fulfilled the following inclusion criteria: *i*) age below 18 years at time of HSCT, *ii*) HSCT performed during 2000–2012, and *iii*) endoscopy with biopsy sampling performed to confirm symptom-based GI-aGVHD within the first year post-HSCT (Figure 7).

Based on the study-specific inclusion criteria, samples were drawn from the primary study population to each study. These study-specific inclusion criteria were:

**Study II**: Available histopathology reports

**Study III**: Available histopathology reports and biopsy slides for RIHA

**Study IV**: Available biopsy slides for RIHA. Lower endoscopy performed with biopsy sampling from at least; the rectosigmoid area, and from the area proximal of the left colonic flexure to the terminal ileum

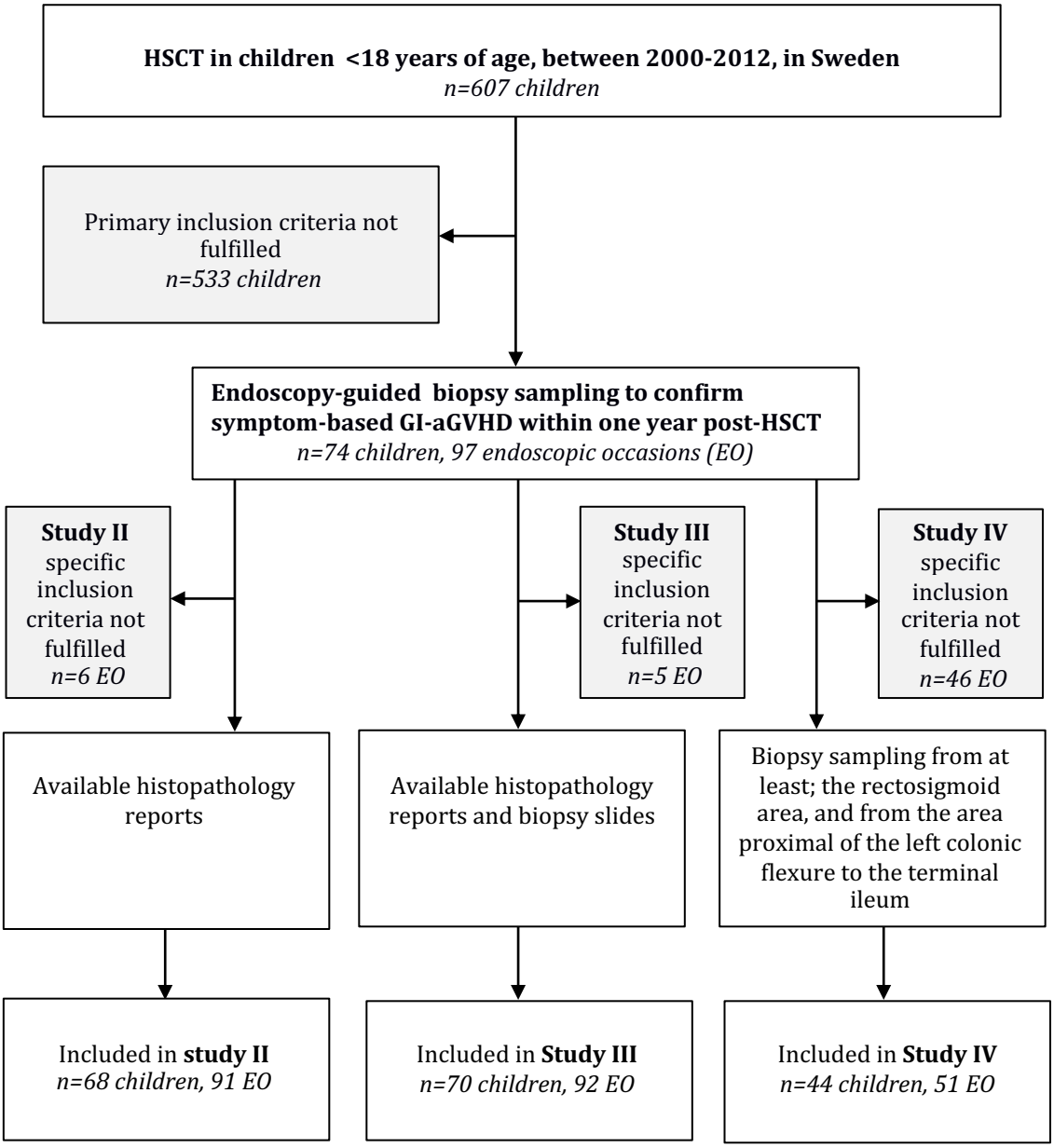


**Figure 7**. Recruitment of participants to study II-IV

## 4.2 METHODS

### 4.2.1 Methods: paper I

This was a retrospective descriptive cohort study based on hospital record data. The study covered day 0–100 post-HSCT. The GI-aGVHD diagnosis was symptom-based and defined and staged by the modified Glucksberg criteria (31). The frequency of GI-aGVHD stages II–IV and other clinical data were compared between children with BuCyMel versus BuCy conditioning.

### 4.2.2 Methods: paper II

This was a retrospective cohort study based on data from histopathology reports and hospital records. Data collection from the hospital records included clinical background information, changes in drug treatment prompted by histopathological findings, two-year post-HSCT survival, and, in those who died, the cause of death. The endoscopic occasions were divided dichotomous, based on a treatment change or not, guided by the histopathology reports.

Treatment changes was defined as initiation, dose escalation, dose reduction or withdrawal of anti-GI-GVHD treatment, and other changes to medication, based on histopathological findings. If data on treatment changes in the medical records were lacking, classification as a treatment change was only done if the change was made in agreement with the histopathology report and was performed subsequent to, but not later than, 14 days after the histopathology report was available.

Due to non-standardized reporting, all endoscopic occasions with a histopathology report indicating a suggestion of GI-GVHD were classified as GI-GVHD. Thus, phrases in the histopathology reports such as “possible,” “slight,” and “minimal” were judged as GI-GVHD.

### 4.2.3 Methods: paper III

This was a retrospective cohort study. The study was based on data from histopathology reports, hospital records, and results from RIHA. The RIHA process is described in paragraph 4.2.5. Histopathological diagnoses established in the CSHA were collected from the histopathology reports. Due to non-standardized reporting, all endoscopic occasions with a histopathology report indicating a suggestion of GI-GVHD were classified as GI-GVHD.

The results from CSHA and RIHA were categorized as GI-GVHD (+) or non-GI-GVHD (-). Based on the results from the CSHA and the RIHA, four subgroups were defined: endoscopic occasions with detection of GI-GVHD in the CSHA and the RIHA (+ +); GI-GVHD solely detected in the RIHA (- +); GI-GVHD detected in the CSHA but reclassified as non-GI-GVHD in the RIHA (+ -); non-GI-GVHD detected in both readings (- -).

Diagnostic disagreement of histopathology-based GI-GVHD diagnosis was based on the subgroup (+ -) and (- +). The analysis of the risk of a subsequent re-endoscopy within one year was based on subgroup affinity (+ +, - +, + -, - -) of the endoscopic occasion that preceded the re-endoscopy. These subgroups were also the basis for the analysis of death within two years post-HSCT. Differences in frequencies of histopathology-based disease severity scores of acute and overlap GI-GVHD were based on endoscopic occasions with GI-GVHD solely detected in the RIHA (- +) versus corresponding cases detected in both assessments (+ +).

### 4.2.4 Methods: paper IV

This was a retrospective study based on results from re-evaluation (hereinafter referred to as RIHA) (Paragraph 4.2.5), and hospital record data. The study was based on the assumption that a finding of GI-GVHD during the RIHA represented a true positive result.

Sensitivity and disagreement analyses were performed. In the disagreement analysis, the results from the RIHA, based on biopsies from different areas of the GI tract were merged into the following groups: *i*) the rectosigmoid area, *ii*) the rest of the colon and the terminal ileum (when intubated), and *iii*) the upper GI tract. The distribution within the GI tract of alternative histopathology-based diagnoses was also based on these groups. In the sensitivity analysis, the results from the RIHA were merged into groups representing different endoscopic procedures: sigmoidoscopy (rectosigmoid area), colonoscopy (the rectum to the cecum), ileocolonoscopy (the rectum to the terminal ileum), and EGD (the duodenum to the esophagus).

### 4.2.5 Common methodology – paper III and IV

The RIHA in *paper III* and *IV* was performed in an identical way; blinded, protocol-based, by one pathologist (A.S.), based on slides with good-quality hematoxylin and eosin and immunohistochemical cytomegalovirus (CMV) staining. The pathologist used the *NIH 2005/NIH 2014* criteria for minimal histological threshold of GI-aGVHD; that is, finding of at least one apoptotic body in a crypt per biopsy piece (8). In cases with histopathology-based acute GI-GVHD or overlap GI-GVHD, histological severity grading was performed in accordance with *Lerner et al.* (139).

In line with *NIH 2005* guidelines, the final RIHA-based GI-GVHD diagnosis in *paper IV* was reported as *possible*, *consistent*, or *definite* GI-GVHD (88), and in *paper III* as *possible* or *likely*, in accordance with the 2014 criteria (8). All endoscopic occasions in *paper III* and *IV* with histological findings indicating GI-GVHD, but other explanations were possible; thus, *possible* GI-GVHD was classified as "normal or non-specific findings." Only endoscopic occasions defined as *consistent/definite* GI-GVHD (88) or *likely* GI-GVHD (8) were judged as GI-GVHD.

During the RIHA, all cases were assessed for CMV as a cause of apoptosis. Separate from the RIHA, the influence of factors other than CMV that may induce apoptosis were evaluated as potential mimickers of GI-GVHD.

### 4.2.6 Definitions

***Paper 1***: *Prolonged regimen-related diarrhea*: Three or more loose or liquid stools per day, without positive infectious disease tests from the feces and with onset during the administration of the conditioning regimen and with duration beyond the time point of neutrophil engraftment.

*Hemorrhagic cystitis*: Occurrence of microscopic or gross hematuria with negative urine bacterial culture (176).

*Sinusoidal obstruction syndrome*: The diagnosis of sinusoidal obstruction syndrome was based on the Jones criteria; that is, bilirubin >34 mM with onset before day 21 post-HSCT together with at least two of the following: *i*) hepatomegaly, *ii*) ascites, *iii*) 5% or greater weight gain (177).

**Paper 2-4**: *Endoscopic occasion*: Any single diagnostic endoscopy procedure, regardless if solely upper or lower endoscopy has been performed or if the occasion included combined upper and lower procedures.

**Paper 3-4**: *Colonoscopy/ileocolonoscopy*: Endoscopy-guided biopsy sampling performed from the area proximal to left colonic flexure to the caecum or terminal ileum (when intubated).

### 4.2.7 Statistics

Endoscopic occasions were the main unit for analyses performed in *paper II-IV*. If serial endoscopic occasions were performed, each occasion was considered independent in the statistical analyses performed in *paper II-III*.

Categorical variables were summarized and presented as frequencies and percentages, and numerical variables as mean, median, standard deviation (SD), and interquartile range (IQR), as appropriate. Fisher's exact was used to compare categorical data between subgroups in the studies. For normally distributed numerical data, the corresponding method was the t-test, and Mann-Whitney U test for data without a normal distribution. These calculations were performed with the exclusion of missing data. The disagreement analyses in *paper III-IV* were based on McNemar's test. For the comparison of the probability of survival between subgroups in *paper II-III* and the probability of a subsequent re-endoscopy in *paper III*, a stratified proportional Cox regression analysis was performed. These analyses were based on endoscopic occasions and each endoscopy, if serial procedures were performed, contributed to the survival function and probability of a subsequent re-endoscopy until the time point of the next endoscopic occasion, but was thereafter censored. Statistical significance was defined as $p<0.05$.

### 4.2.8 Ethics

All studies included in this thesis were conducted in accordance with the Declaration of Helsinki and were approved by the Swedish Ethical Review Authority, Sweden.

# 5 RESULTS AND DISCUSSION

## 5.1 RESULTS AND DISCUSSION PAPER I

Twenty-five children were enrolled, seventeen conditioned with BuCyMel and eight with BuCy.

Key finding:

- Forty-seven percent (8/17) of the children that received addition of Mel to the BuCy conditioning, versus none (0/8) in the BuCy group, developed symptom-based GI-aGVHD (stages 2-4) ($p<0.05$)

Other findings:

- Overall survival, independently of time post-HSCT, was 53 % (9/17) in the BuCyMel group and 63 % (5/8) in the BuCy group
- Relapse of the underlying diagnosis for HSCT occurred in four patients, two in the BuCyMel group and two in the BuCy group
- Toxic manifestations, i.e., hemorrhagic cystitis, prolonged regimen-related diarrhea and sinusoidal obstruction syndrome, were observed in 47 % (8/17), 35 % (6/17), and 6 % (1/17), respectively, in the BuCyMel group. None of the children in the BuCy group developed these manifestations.

The rationale of adding Mel to the BuCy conditioning regimen in children has been to reduce the risk of relapse (113). However, Mel has also been associated with gastrointestinal toxicity (178), thereby potentially acting as initiator of aGVHD (43, 44, 179).

The results in *paper I* indicate that the addition of Mel to the BuCy conditioning increases the risk of regimen-related toxicity and the risk of GI-aGVHD (stages 2-4). However, the role of adding Mel to the BuCy conditioning regimen needs to be considered in a broader context. A previous study that included children with acute myeloid leukemia showed that BuCyMel, compared to BuCy conditioning, was associated with a higher probability of survival, a lower relapse rate, and a higher risk of severe aGVHD (grade 3–4) (119). In that study, the aGVHD diagnosis was symptom-based, thus less specific (7), and no specification was given of the incidence of aGVHD in each affected organ system for the BuCy and the BuCyMel groups (119).

Due to the small sample size in *study I*, the probability of survival and risk of relapse needs to be further clarified. Preferably, that type of study would have a prospective randomized design and would include endoscopy with histopathological assessment for all individuals developing clinically suspected GI-aGVHD.

## 5.2 RESULTS AND DISCUSSION PAPER II

Ninety-one endoscopic occasions performed in sixty-eight children were enrolled. Twenty-three procedures were re-endoscopies. Anti-GI-GVHD treatment at the time of the endoscopic occasion was ongoing in 71% (65/91) and not ongoing in 29 % (26/91).

Key findings:

- Treatment changes in response to histopathology reports was observed in 48 % (44/91)
- In 18 % (12/65) with start of anti-GI-GVHD treatment pre-endoscopically, no medical causes were found justifying that treatment
- The two-year overall post-HSCT survival was 62 %, without difference between the treatment changes versus the unchanged treatment group (Hazard ratio 1.28, 95% CI 0.60-2.70, p=0.524)

Other findings:

- The negative prognostic factor, grossly bloody diarrhea, was present in 27 % (12/44) in the treatment change group and in 9 % (4/47) in the unchanged treatment group (p=0.003), at the time of endoscopy

In *paper II*, almost every second endoscopic occasion performed to confirm symptom-based GI-aGVHD was followed by treatment changes based on the histopathology assessment. The corresponding figure was 45 % in an adult patient study, using a similar definition of treatment change as in *paper II* (180). In the only previous pediatric study, 30 % of the endoscopic occasions were followed by initiation or dose escalation of anti-GI-GVHD drugs (173). These results support that treatment decisions in daily clinical practice are influenced by endoscopy-guided histopathological assessment.

In almost every fifth endoscopic occasion where anti-GI-GVHD treatment was started pre-endoscopically, no histopathological evidence of GI-GVHD or other medical causes were found to justify that treatment. Instead, in these endoscopic occasions, the anti-GI-GVHD treatment exposed children unnecessarily to the risk of avoidable side effects associated with immunosuppressive drugs (169-171).

In *paper II*, no difference was found in the overall two-year post-HSCT survival in individuals in the treatment change group compared with individuals in the unchanged treatment group. This result was unexpected since: *i*) the negative prognostic factor, grossly bloody diarrhea (181, 182), was more frequently observed in the treatment change group and, *ii*) all individuals in the study cohort exposed to overtreatment or undertreatment with anti-GI-GVHD agents pre-endoscopically belonged, by definition, to the treatment change group. Thus, the treatment change group possibly represents individuals in *study II* with less favorable survival chances post-HSCT. Based on that, the similar two-year overall survival in the treatment change group and the unchanged treatment group, may imply a positive influence of histopathology-guided treatment changes on survival in children with clinically suspected GI-GVHD.

## 5.3 RESULTS AND DISCUSSION PAPER III

Ninety-two endoscopic occasions performed in seventy children were enrolled. Twenty-two procedures were re-endoscopies. In the analyses of risk of a subsequent re-endoscopy within one-year and of death within two-year post-HSCT, the subgroup (+ -) was excluded due to its small sample size (three endoscopic occasions).

Key findings:

- In the *NIH*-based RIHA, histopathology-based GI-GVHD diagnosis was established in 67 of 92 (73 %) of the endoscopic occasions, and in the CSHA, in 50 of 92 (54 %) (p=0.014)
- In 94 % (47/50), the RIHA confirmed the GI-GVHD diagnosis established in the CSHA (+ +). The corresponding result for non-GI-GVHD (- -) was 52 % (22/42)
- Diagnostic disagreement, i.e., GI-GVHD solely detected in the RIHA (- +) or GI-GVHD detected in the CSHA but not in the RIHA (+ -), was observed in 48 % (20/42) and 6 % (3/50), respectively (McNemar´s test, p=0.0008)
- The risk of a subsequent re-endoscopy within one-year post-HSCT was higher in endoscopic occasions with GI-GVHD solely detected in the RIHA (- +) than if non-GI-GVHD was detected in both assessments (- -) (P=0.005)

Other findings:

- The results were not influenced by factors, other than GI-GVHD, with the potential to induce apoptosis in the GI tract
- GI-GVHD solely detected in the RIHA, and consequently undetected in the CSHA (- +), had no impact on the probability of survival
- Histopathology-based severity grade 1 was observed in 70 % (14/20) of the endoscopic occasions with GI-GVHD solely detected in the RIHA (- +) and in 34 % (15/45) of the endoscopic occasions with GI-GVHD detected in both assessments (+ +) (p=0.008)

In *paper III*, the GI-GVHD diagnosis was more frequently established in the *NIH 2014*-based RIHA versus CSHA. Furthermore, the assessment performed by the pathologist in the RIHA showed no indication of being misled by drugs or infectious agents with the potential to induce apoptosis, thereby mimicking the histological pattern of GI-GVHD.

Two of three cases with non-detected GI-GVHD in the CSHA but detected in the RIHA (- +) had histological severity grade 1, i.e., solely finding crypt apoptosis (139). This result indicates that some pathologists performing the CSHA required a higher number of apoptotic bodies to establish the GI-GVHD diagnosis versus the minimal criteria for histopathology-based GI-GVHD by the *NIH* 2014 (8).

The *MAGIC* consensus GVHD research guidelines point out histopathology as the most important diagnostic test for attributing gastrointestinal symptoms to GI-GVHD (7). However, the clinical usefulness of that statement is negatively influenced by the lack of consensus

regarding minimal criteria of the histopathology-based GI-GVHD diagnosis. In the absence of a diagnostic reference standard, other markers to evaluate the post-test versus pre-test probability of a correct diagnosis are needed (163). In *paper III*, GI-GVHD solely detected in the RIHA (- +) did not influence the probability of two-year post-HSCT survival. However, the risk of a subsequent re-endoscopy was higher if GI-GVHD was detected in the RIHA but not in the CSHA (- +) versus if GI-GVHD was not detected in any of the assessment methods (- -). The increased risk of a subsequent re-endoscopy in the - + subgroup indicated a potential advantage of using the *NIH 2014* criteria in children with symptom-based GI-aGVHD but without a histopathology-based confirmation in the CSHA.

## 5.4 RESULTS AND DISCUSSION PAPER IV

Fifty-one endoscopic occasions performed in 44 children were enrolled.

Key findings:

- In 76 % (39/51) of the endoscopic occasions, a histopathology-based GI-GVHD diagnosis was established
- The sensitivity for the GI-GVHD diagnosis was 85 % for biopsies from the rectosigmoid area
- The sensitivity of histopathology-based GI-GVHD diagnosis in combined biopsy sampling from the rectosigmoid area and the upper GI tract was 97 %, which was similar to biopsies collected from rectosigmoid-ileocolonic areas
- McNemar's test revealed a difference between non-detected histopathology-based GI-GVHD diagnosis in biopsies collected from the rectosigmoid area versus detected elsewhere in the GI tract (p=0.031)

Other findings:

- Immunohistochemical detection of CMV was the most frequently observed alternative diagnosis found in 14 % (7/51) of the endoscopic occasions
- In 6/7 cases with immunohistochemical detection of CMV, concomitant GI-GVHD was present
- CMV had a patchy distribution in the GI tract, with 2/7 detected in biopsies from the rectosigmoid area. The corresponding figure was 6/7 in merged analysis of biopsies from the rectosigmoid and the upper GI tract

Sigmoidoscopy represents a straightforward endoscopic procedure that, in a pediatric population, might be performed under minimal sedation (141-143). Thus, sigmoidoscopy has the potential to be performed as soon as GI symptoms appears, potentially allowing early distinction between GI-GVHD and other GI tract conditions that may require different treatment.

In *paper IV*, biopsy sampling from the rectosigmoid area detected 85 % of all cases with histopathology-based GI-GVHD diagnosis. Similar results have been observed in previous studies (131, 144). However, *paper 4* is the first pediatric study to report a statistically significant difference between non-detected GI-GVHD in biopsies collected from the rectosigmoid area versus detected elsewhere in the GI tract. Furthermore, biopsies from the rectosigmoid area were less reliable for detection of alternative diagnoses, such as CMV. Thus, full upper and lower endoscopy; combined upper endoscopy and sigmoidoscopy; or colonoscopy/ileocolonoscopy alone should be considered as procedures of choice in children with clinically suspected GI-GVHD.

## 5.5 METHODOLOGICAL CONSIDERATIONS

### 5.5.1 Strengths

- In the context of HSCT, the most substantial difference between children and adult patients is related to differences in underlying diagnoses for the transplantation (10). Despite that, studies conducted specifically in pediatric HSCT patients are far less as common as corresponding studies including adult HSCT patients (183). All studies performed in this thesis included only children below 18 years of age.
- In *paper I*, the influence on the result of chemotherapy given prior to HSCT was minimized since only children with JMML and MDS were included.
- The study cohorts in *paper II-IV* only included children with endoscopy performed within one-year post-HSCT. That time limitation was used to avoid mixed indications for the endoscopic procedures (e.g. clinically suspected GI-aGVHD together with another indication).

### 5.5.2 Weaknesses

- The retrospective designs and limited sample sizes may have influenced the outcomes in all studies.
- In *paper I*, the classification of the GI-aGVHD diagnosis was symptom-based and therefore less specific as compared to histopathology-based diagnosis (7).
- In *paper II*, the vast majority had a note in the medical records indicating the motivation for the treatment change. However, potential misclassification may have occurred in those cases without this type of note.
- The multicenter design of Study II may have influenced the results from CSHA due to interobserver and interinstitutional differences in the assessment of the GI-GVHD diagnosis (132, 134).
- The RIHA in study III-IV was performed in a scientific setting, as a protocol-based procedure, by one pathologist. Thus, the setting differed from that of daily clinical practice.

# 6 CONCLUSIONS

***Paper I***: The conditioning regimen of BuCyMel, compared with BuCy, resulted in an increased incidence of GI-aGVHD (stages 2-4) within 100 days post-HSCT in HSCT-treated children with JMML and MDS. However, the role of adding Mel to the BuCy conditioning regimen needs to be considered in a broader context, including the risk of relapse and probability of survival.

***Paper II***: Endoscopy with histopathological assessment was found to influence the treatment decisions and should therefore be considered in children with suspected GI-aGVHD.

***Paper III***: In children with symptom-based GI-aGVHD but without a CSHA-based confirmation of the diagnosis, a second histopathological assessment based on the *NIH 2014* criteria should be considered before performing a re-endoscopy.

***Paper IV***: Sigmoidoscopy combined with upper endoscopy, colonoscopy/ileocolonoscopy, and full upper and lower endoscopy appear equally reliable for detecting GI-GVHD and should therefore be regarded as the preferred choices for an endoscopic procedure in children with clinically suspected GI-GVHD. However, in severely ill children with contraindications for general anesthesia or extensive endoscopy, sigmoidoscopy represents an acceptable option.

# 7 REFLECTIONS AND FUTURE PERSPECTIVES

More than 50 years have passed since the first endoscopic procedure was performed in a child due to clinically suspected GI-GVHD. However, we are still dealing today with basic questions regarding the diagnosis of GI-GVHD in pediatric patients treated with HSCT.

A striking finding in *paper II-IV* was that anti-GI-GVHD treatment was ongoing in the majority of the children at time point of the endoscopy. In fact, the anti-GI-GVHD treatments had been started approximately 13 days pre-endoscopically. One might argue that endoscopies performed under these circumstances are not true primary diagnostic procedures; rather, they are treatment controls or are performed to detect alternative diagnoses to GI-GVHD. Whether the lag time between the start of anti-GI-GVHD treatment and endoscopy was intentional is not known. However, causes and effects need to be clarified if the ambition is to switch to "true" diagnostic endoscopies in the future. A good start would be to initiate a survey highlighting the current clinical practice of diagnosis of GI-aGVHD in children in Sweden.

Isolated upper GI tract aGVHD has been estimated to affect approximately 3 % of the adult patients undergoing HSCT (184). This manifestation of aGVHD, has been associated with a response rate to glucocorticoids of approximately 90 % (82, 83) and with an overall survival similar to that of patients without aGVHD (184). Based on these findings, a re-classification of isolated upper GI tract aGVHD from aGVHD grade 2 to aGVHD grade 0–1 has been proposed (184). Since this type of re-classification may have implications regarding the choice of anti-GVHD treatment, isolated upper GI tract aGVHD represents an important topic of research also in pediatric HSCT patients.

A growing number of publications are reporting that oral/enteral nutrition versus parenteral nutrition reduces the risk of development of GI-GVHD (110, 185). These results are in line with the concept that at least minimal enteral nutrition is beneficial for the GI tract (111). However, enteral nutrition as a treatment option has not been studied systematically in the context of pediatric GI-GVHD, even though one case report has been published (186). In pediatric Crohn disease (PCD), exclusively enteral nutrition is an established treatment modality with a clinical response rate equally as good as treatment with 1,6 mg methylprednisolone per kilogram bodyweight/day (187). Evaluation of the applicability of the knowledge derived from enteral nutrition treatment of PCD in pediatric GI-GVHD constitutes an important future research question.

Dysbiosis has been associated with an increased risk for the development of GI-GVHD (69), and the lowest microbial diversity has been observed during the first month post-HSCT (69, 188). Consequently, a potential strategy to prevent the development of GI-aGVHD might be prophylactic administration of probiotics or consideration of fecal microbiota transplantation (FMT) from healthy donors at the time of HSCT. Probiotics administered to children during the HSCT process have been considered safe (189). Furthermore, a placebo-controlled pediatric trial is currently ongoing to assess the efficacy of *Lactobacillus plantarum* in preventing the development of GI-aGVHD and CDI (*ClinicalTrials.gov*, NCT03057054).

Regarding FMT, different aspects of safety in the HSCT setting and the choice of method to perform the transplantation need clarification. At *ClinicalTrials.gov*, (August 18, 2020), using the search terms "gvhd and microbiota", eleven out of thirty search results were registered trials with the objective of evaluating FMT as a treatment for severe aGVHD, most often GI-GVHD. However, no trials were found with the objective of evaluating the incidence of GI-aGVHD in prophylactic FMT administration.

Probably the most important issue for the near future, from a clinical and a scientific viewpoint, is to continue to introduce a "common language" within the HSCT community. A "common language" with uniform definitions is a requirement for comparability of results from different studies. App-based diagnosis and grading of clinical GVHD, eGVHD, represents a strategy for establishing a "common language". It was introduced approximately two years ago, with good preliminary results (190). Hopefully, a common histopathological language with uniform definition of the threshold of histopathology-based GI-GVHD is next in line, to create uniformity within the HSCT community.

# 8 ACKNOWLEDGEMENTS

To all the children that have participated in my studies, this thesis is as much yours as it is mine!!

During my PhD process, five persons have been fundamentally important in getting the process rolling. Anna, my dear "almost wife," you have contributed with outstanding support, positive input, and unconditional love all along the road. Kärlek! Britt and Thomas C, my dear friends and supervisors. You have opened the door to the world of science for me and carefully guided me on my way. My deepest respect and gratitude for all that you have done for me! Attila, the outstanding pathologist who has introduced me to the fascinating world of histopathology and who has performed the extensive work of re-assessment of all the biopsy slides included in this thesis. Attila, you are a Hero! Andreas, my dear brother and co-supervisor, the latter without knowing it! Andreas, many thanks for good advice, support, and for sharing your knowledge with me!

Britt and Thomas (again), Moustapha and Jonas, my official supervisors, I owe you the greatest gratitude for all your support, help, and guidance during my PhD process! Many thanks! Moustapha, special thanks for being an unlimited source of inspiration and for many good advices, throughout my training! And Moustapha, thanks for countless laughs together!

I have had the privilege of having the head of Division of Pediatrics, CLINTEC, as mentor. Björn, once upon a time, you employed me at the Department of Pediatric Gastroenterology, Hepatology and Nutrition at Karolinska University Hospital. I don't think any of us, at that time, could have predicted that the most solid outcome of that employment would be this thesis! As my boss and mentor, I have never stopped being deeply impressed by the positive atmosphere that you create around you, your clear thinking, and your care for people in general and for me, in particular. Thanks, Björn!

Many thanks to colleagues, friends, and co-writers at the non-Stockholm pediatric HSCT centers in Sweden who have participated and contributed to this thesis: Karin and Tom in Gothenburg, Jacek in Lund and Johan in Uppsala! Thanks for your co-operation and support!

Thanks to Claude and Mats at CLINTEC for believing in me!

Special thanks to my current and former boss at Sodertalje Hospital, Henrik and Sven, as well as to Mia. Henrik, Sven, and Mia, your flexibility, support, and overall consideration for me has been extremely important during this process! Many many thanks!

Thilda, my angel and daughter, my deepest gratitude for your love and support!

My gastro dream-team members at Sodertalje Hospital! First of all, Peter, my friend, soulmate, and (almost) roommate at Sodertalje Hospital. Thanks for always showing great interest in my work, for your support, and for all your help with the care of my patients when I have not been in charge! Also, many thanks to the other members of the dream team: Annika, Yvonne, and

Jan! Bettan, you are not forgotten! Many thanks to Liz and to all other coworkers, at Sodertalje Hospital.

The Britt team: Emma, Kristin, Lena, and Sara. Thanks to all of you for fruitful discussions and inspiration. Furthermore, to the oracle among PhD students at CLINTEC, Markus, thanks for your support and for sharing your knowledge with me!

To my family and friends who never gave up on me! Thanks!

Last but not least, many thanks to the staff at CLINTEC, to Stephan and all others at CAST, to colleagues and friends at the Division of Pediatric Gastroenterology and the Division of Pediatric Hematology, Immunology and HCT, Karolinska University Hospital, and finally to Claude, Anneli, Pär, and Peo, and all the other co-workers at Children's Hospital Martina.

## 8.1 FINANCIAL SUPPORT

Studies included in this PhD project have received financial support from:

- The Swedish Childhood Cancer Foundation
- The Stockholm County Council Research Foundation (ALF)
- Swedish Research Council (VR)
- The Swedish Order of Freemasons
- The Samariten Foundation for Paediatric Research
- The Mary Béves Foundation

# 9 SAMMANFATTNING PÅ SVENSKA

## 9.1 BAKGRUND

Cirka 40–50 barn (<18 år) i Sverige per år genomgår stamcellstransplantation. Blodcancer (leukemi) är den enskilt vanligaste orsaken till behandlingen. Vid en stamcellstransplantation förbehandlas, konditioneras, patienten med olika cellgifter, ibland även strålning, följt av att friska blod-stamceller från en annan person överförs. Hos mer än hälften av alla barn som genomgår stamcellstransplantation kommer givarens immunceller uppfatta den nya kroppen som "främmande". Den sjukdom som då uppstår heter givare-mot-värd sjukdom (GVHD) och innebär att givarens immunceller angriper och skadar organ hos mottagaren. Vanligast är att GVHD drabbar huden, följt av magtarmkanalen och levern.

Cirka vart fjärde barn (<18 år) som genomgår stamcellstransplantation drabbas av GVHD i magtarmkanalen (MT-GVHD). Diagnostiken av MT-GVHD baseras i första hand på symptom såsom; diarré, viktnedgång, ont i magen, illamående och kräkningar. Emellertid, då dessa symptom är ospecifika och alltså förekommer vid en rad andra mag-tarmsjukdomar rekommenderas att slutgiltig MT-GVHD diagnos baseras på endoskopi (undersökning med kikarinstrument) av magtarmkanalen samtidigt som provbitar (biopsier) tas för en efterföljande mikroskopisk undersökning.

Behandling av MT-GVHD, utgörs av kortison. Tyvärr är kortisonbehandling behäftad med risker, så som infektioner, högt blodsocker och högt blodtryck. Vid uteblivet behandlingssvar på kortison, halveras den förväntade två-årsöverlevnaden efter transplantationen.

Denna avhandling syftar till: *i*) att jämföra två olika konditioneringar inför stamcellstransplantation avseende insjuknande in MT-GVHD, och *ii*) att utvärdera olika kliniska aspekter av mikroskopiskt baserad diagnostik av MT-GVHD hos barn.

## 9.2 MATERIAL OCH METOD

Samtliga studier som ingår i denna avhandlingen har haft en tillbakablickande - retrospektiv design. Studie 1 har inkluderat barn och ungdomar (<18 år) som transplanterats vid Karolinska Universitetssjukhuset Huddinge, mellan år 2000-2010, på grund av juvenil myelomonocyt leukemi (JMML) eller myelodysplastiskt syndrom (MDS) och som konditionerats med busulfan och cyklofosfamid, +/- melfalan. Till studie 2-4 inkluderades barn och ungdomar (<18 år) som genomgått stamcellstransplantation vid något transplantationscenter i Sverige mellan år 2000-2012 och som dessutom, inom ett år efter transplantationen, genomgått endoskopi med biopsitagning på misstanke om MT-GVHD. Inkludering till studie 4 förutsatte dessutom att minst en biopsi tagits från ändtarm/nedre del av tjocktarm (sigmoideum), samt minst en från den uppåtstigande eller tvärgående delen av tjocktarmen. I studie 3-4 genomfördes en förnyad, standardiserad mikroskopisk undersökning av samtliga biopsier. Denna undersökning utfördes av en patolog och var baserad på riktlinjer från amerikanska *National Institutes of Health (NIH) 2014* avseende minimi-kriterier för mikroskopisk baserad

MT-GVHD diagnos. I studie 3 jämfördes diagnoser fastställda vid den förnyade standardiserade mikroskopiska undersökningen med diagnoser som fastställdes vid den kliniska rutinmässiga mikroskopiska undersökningen.

## 9.3 RESULTAT

*Studie 1*: Tjugofem barn ingick i denna studie. Hos 44 % (8/17) som konditionerades med busulfan, cyklofosfamid och melfalan jämfört ingen (0/8) som konditionerades med busulfan och cyklofosfamid, utvecklades måttlig till svår MT-GVHD inom 100 dagar efter transplantationen ($p<0.05$). *Studie 2*: Sextioåtta barn med 91 endoskopitillfällen inkluderades. Hos 48 % (44/91), noterades förändringar i läkemedelsbehandlingen baserat på svar från klinisk rutinmässig mikroskopisk undersökning. *Studie 3:* Sjuttio barn med 92 endoskopitillfällen inkluderades. MT-GVHD observerades efter 67 av 92 (73 %) endoskopitillfällen vid förnyad, standardiserad mikroskopisk undersökning och efter 50 av 92 (54 %) endoskopitillfällen vid klinisk rutinmässig mikroskopisk undersökning ($p = 0,014$). Risken för en efterföljande ny endoskopi var högre om MT-GVHD enbart påvisades i den förnyade, standardiserade mikroskopiska undersökningen, jämfört med om vare sig klinisk rutinmässig eller standardiserad mikroskopisk undersökning påvisade MT-GVHD ($p=0,005$). *Studie 4*: Fyrtiofyra barn med 51 endoskopitillfällen deltog. MT-GVHD, baserad på förnyad, standardiserad mikroskopisk undersökning observerades vid 76 % (39/51) av endoskopiska tillfällena. I biopsier från ändtarm och nedre tjocktarm kunde förnyad, standardiserad mikroskopisk undersökning påvisa 85 % av alla mikroskopiskt fastställda fall av MT-GVHD. Motsvarande siffra för kombinerad biopsitagning från ändtarm och nedre tjocktarmen tillsammans med biopsier från övre magtarmkanalen var 97 %, vilket var samma resultat som om ändtarm, hela tjocktarmen och sista del av tunntarmen biopserades.

## 9.4 SLUTSATSER

- Tillägg av melfalan till busulfan och cyklofosfamid konditionering hos barn med JMML eller MDS ökar risken för utveckling av MT-GVHD
- Endoskopi följt av mikroskopisk undersökning av insamlade magtarm-biopsier har en inverkan på valet av läkemedelsbehandling, och kan därför rekommenderas
- Hos barn med symptom på MT-GVHD där klinisk rutinmässig mikroskopisk undersökning inte påvisat sjukdomen, bör en förnyad mikroskopisk undersökning, baserad på *NIH 2014*-kriterierna för MT-GVHD genomföras innan en ny endoskopi utförs
- Endoskopi med biopsitagning vid misstänkt MT-GVHD hos barn bör omfatta antingen full övre och nedre endoskopi, endoskopi av ändtarm och nedre tjocktarm kombinerat med övre endoskopi eller full nedre endoskopi

# 10 REFERENCES


1. Vos O, Davids JA, Weyzen WW, Van Bekkum DW. Evidence for the cellular hypothesis in radiation protection by bone marrow cells. Acta Physiol Pharmacol Neerl. 1956;4(4):482-6.
2. Barnes DW, Corp MJ, Loutit JF, Neal FE. Treatment of murine leukaemia with X rays and homologous bone marrow; preliminary communication. Br Med J. 1956;2(4993):626-7.
3. van Bekkum DW dVM. Radiation Chimeras. London: Logo- s/Academic Press; 1967.
4. Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet. 1968;2(7583):1364-6.
5. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet. 1968;2(7583):1366-9.
6. Gilger MA. Gastroenterologic endoscopy in children: past, present, and future. Curr Opin Pediatr. 2001;13(5):429-34.
7. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transplant. 2016;22(1):4-10.
8. Shulman HM, Cardona DM, Greenson JK, Hingorani S, Horn T, Huber E, et al. NIH Consensus development project on criteria for clinical trials in chronic graft-versus-host disease: II. The 2014 Pathology Working Group Report. Biol Blood Marrow Transplant. 2015;21(4):589-603.
9. Carpenter PA, Macmillan ML. Management of acute graft-versus-host disease in children. Pediatr Clin North Am. 2010;57(1):273-95.
10. Passweg JR, Baldomero H, Peters C, Gaspar HB, Cesaro S, Dreger P, et al. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. Bone Marrow Transplant. 2014;49(6):744-50.
11. Svenberg P, Remberger M, Uzunel M, Mattsson J, Gustafsson B, Fjaertoft G, et al. Improved overall survival for pediatric patients undergoing allogeneic hematopoietic stem cell transplantation - A comparison of the last two decades. Pediatr Transplant. 2016;20(5):667-74.
12. Vaht K, Göransson M, Carlson K, Isaksson C, Lenhoff S, Sandstedt A, et al. Incidence and outcome of acquired aplastic anemia: real-world data from patients diagnosed in Sweden from 2000-2011. Haematologica. 2017;102(10):1683-90.
13. Kollman C, Spellman SR, Zhang MJ, Hassebroek A, Anasetti C, Antin JH, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016;127(2):260-7.
14. Flowers ME, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood. 2011;117(11):3214-9.
15. Sano H, Hilinski JA, Qayed M, Applegate K, Newton JG, Watkins B, et al. Early blood stream infection following allogeneic hematopoietic stem cell transplantation is a risk factor for acute grade III-IV GVHD in children and adolescents. Pediatr Blood Cancer. 2018;65(2).

16. Ayuk F, Balduzzi A. Donor Selection for Adults and Pediatrics. In: Carreras E, Dufour C, Mohty M, Kröger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. Cham (CH): Springer
Copyright 2019, EBMT and the Author(s). 2019. p. 87-97.
17. de Koning C, Nierkens S, Boelens JJ. Strategies before, during, and after hematopoietic cell transplantation to improve T-cell immune reconstitution. Blood. 2016;128(23):2607-15.
18. D'Souza A, Fretham C, Lee SJ, Arora M, Brunner J, Chhabra S, et al. Current Use of and Trends in Hematopoietic Cell Transplantation in the United States. Biol Blood Marrow Transplant. 2020;26(8):e177-e82.
19. Nagler A, Shimoni A. Conditioning. In: Carreras E, Dufour C, Mohty M, Kröger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. Cham (CH): Springer
Copyright 2019, EBMT and the Author(s). 2019. p. 99-107.
20. Blazar BR, Hill GR, Murphy WJ. Dissecting the biology of allogeneic HSCT to enhance the GvT effect whilst minimizing GvHD. Nat Rev Clin Oncol. 2020.
21. Lawitschka A, Lucchini G, Strahm B, Dalle JH, Balduzzi A, Gibson B, et al. Pediatric acute graft-versus-host disease prophylaxis and treatment: surveyed real-life approach reveals dissimilarities compared to published recommendations. Transpl Int. 2020.
22. Jaiswal SR, Chakrabarti A, Chatterjee S, Bhargava S, Ray K, O'Donnell P, et al. Haploidentical Peripheral Blood Stem Cell Transplantation with Post-Transplantation Cyclophosphamide in Children with Advanced Acute Leukemia with Fludarabine-, Busulfan-, and Melphalan-Based Conditioning. Biol Blood Marrow Transplant. 2016;22(3):499-504.
23. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol. 2016;7:507.
24. Ruutu T, Eriksson B, Remes K, Juvonen E, Volin L, Remberger M, et al. Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. Blood. 2002;100(6):1977-83.
25. Alexandersson A, Koskenvuo M, Tiderman A, Lääperi M, Huttunen P, Saarinen-Pihkala U, et al. Viral infections and immune reconstitution interaction after pediatric allogenic hematopoietic stem cell transplantation. Infect Dis (Lond). 2019;51(10):772-8.
26. Kim HT, Armand P. Clinical endpoints in allogeneic hematopoietic stem cell transplantation studies: the cost of freedom. Biol Blood Marrow Transplant. 2013;19(6):860-6.
27. Champlin RE, Schmitz N, Horowitz MM, Chapuis B, Chopra R, Cornelissen JJ, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). Blood. 2000;95(12):3702-9.
28. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.e1.
29. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945-56.

30. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18(4):295-304.
31. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825-8.
32. Schoemans HM, Lee SJ, Ferrara JL, Wolff D, Levine JE, Schultz KR, et al. EBMT-NIH-CIBMTR Task Force position statement on standardized terminology & guidance for graft-versus-host disease assessment. Bone Marrow Transplant. 2018;53(11):1401-15.
33. Kato M, Kurata M, Kanda J, Kato K, Tomizawa D, Kudo K, et al. Impact of graft-versus-host disease on relapse and survival after allogeneic stem cell transplantation for pediatric leukemia. Bone Marrow Transplant. 2019;54(1):68-75.
34. Brissot E, Rialland F, Cahu X, Strullu M, Corradini N, Thomas C, et al. Improvement of overall survival after allogeneic hematopoietic stem cell transplantation for children and adolescents: a three-decade experience of a single institution. Bone Marrow Transplant. 2016;51(2):267-72.
35. Faraci M, Caviglia I, Biral E, Morreale G, Giardino S, Garbarino L, et al. Acute graft-versus-host disease in pediatric allogeneic hematopoietic stem cell transplantation. Single-center experience during 10 yr. Pediatr Transplant. 2012;16(8):887-93.
36. Goussetis E, Paisiou A, Kitra V, Peristeri I, Vessalas G, Stefanaki K, et al. Acute gastrointestinal graft-versus-host disease in pediatric patients: serum albumin on day 5 from initiation of therapy correlates with nonrelapse mortality and overall survival. Biol Blood Marrow Transplant. 2011;17(7):1058-66.
37. Barker CC, Anderson RA, Sauve RS, Butzner JD. GI complications in pediatric patients post-BMT. Bone Marrow Transplant. 2005;36(1):51-8.
38. Alsultan A, Giller RH, Gao D, Bathurst J, Hild E, Gore L, et al. GVHD after unrelated cord blood transplant in children: characteristics, severity, risk factors and influence on outcome. Bone Marrow Transplant. 2011;46(5):668-75.
39. Qayed M, Wang T, Hemmer MT, Spellman S, Arora M, Couriel D, et al. Influence of Age on Acute and Chronic GVHD in Children Undergoing HLA-Identical Sibling Bone Marrow Transplantation for Acute Leukemia: Implications for Prophylaxis. Biol Blood Marrow Transplant. 2018;24(3):521-8.
40. Keesler DA, St Martin A, Bonfim C, Seber A, Zhang MJ, Eapen M. Bone Marrow versus Peripheral Blood from Unrelated Donors for Children and Adolescents with Acute Leukemia. Biol Blood Marrow Transplant. 2018;24(12):2487-92.
41. Mattsson J, Westin S, Edlund S, Remberger M. Poor oral nutrition after allogeneic stem cell transplantation correlates significantly with severe graft-versus-host disease. Bone Marrow Transplant. 2006;38(9):629-33.
42. Azarnoush S, Bruno B, Beghin L, Guimber D, Nelken B, Yakoub-Agha I, et al. Enteral nutrition: a first option for nutritional support of children following allo-SCT? Bone Marrow Transplant. 2012;47(9):1191-5.
43. Liu D, Yan C, Xu L, Wang Y, Han W, Zhang X, et al. Diarrhea during the conditioning regimen is correlated with the occurrence of severe acute graft-versus-host disease through systemic release of inflammatory cytokines. Biol Blood Marrow Transplant. 2010;16(11):1567-75.
44. Johansson JE, Ekman T. Gut toxicity during hemopoietic stem cell transplantation may predict acute graft-versus-host disease severity in patients. Dig Dis Sci. 2007;52(9):2340-5.

45. Takashima S, Kadowaki M, Aoyama K, Koyama M, Oshima T, Tomizuka K, et al. The Wnt agonist R-spondin1 regulates systemic graft-versus-host disease by protecting intestinal stem cells. J Exp Med. 2011;208(2):285-94.
46. Nakasone H, Fukuda T, Kanda J, Mori T, Yano S, Kobayashi T, et al. Impact of conditioning intensity and TBI on acute GVHD after hematopoietic cell transplantation. Bone Marrow Transplant. 2015;50(4):559-65.
47. Lee SJ. Classification systems for chronic graft-versus-host disease. Blood. 2017;129(1):30-7.
48. Cuvelier GDE, Nemecek ER, Wahlstrom JT, Kitko CL, Lewis VA, Schechter T, et al. Benefits and challenges with diagnosing chronic and late acute GVHD in children using the NIH consensus criteria. Blood. 2019;134(3):304-16.
49. Lee JW, Lee DH, Jang PS, Yi MS, Chung NG, Cho B, et al. Prognostic implications of the NIH consensus criteria in children with chronic graft-versus-host disease. Yonsei Med J. 2011;52(5):779-86.
50. Felix NJ, Allen PM. Specificity of T-cell alloreactivity. Nat Rev Immunol. 2007;7(12):942-53.
51. Spierings E, Fleischhauer K. Histocompatibility. In: th, Carreras E, Dufour C, Mohty M, Kroger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. Cham (CH): Springer
Copyright 2019, EBMT and the Author(s). 2019. p. 61-8.
52. Paczesny S, Hanauer D, Sun Y, Reddy P. New perspectives on the biology of acute GVHD. Bone Marrow Transplant. 2010;45(1):1-11.
53. Spierings E. Minor histocompatibility antigens: past, present, and future. Tissue Antigens. 2014;84(4):374-60.
54. Randolph SS, Gooley TA, Warren EH, Appelbaum FR, Riddell SR. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. Blood. 2004;103(1):347-52.
55. Stern M, Brand R, de Witte T, Sureda A, Rocha V, Passweg J, et al. Female-versus-male alloreactivity as a model for minor histocompatibility antigens in hematopoietic stem cell transplantation. Am J Transplant. 2008;8(10):2149-57.
56. Billingham RE. The biology of graft-versus-host reactions. Harvey Lect. 1966;62:21-78.
57. Socie G, Blazar BR. Acute graft-versus-host disease: from the bench to the bedside. Blood. 2009;114(20):4327-36.
58. Mir E, Palomo M, Rovira M, Pereira A, Escolar G, Penack O, et al. Endothelial damage is aggravated in acute GvHD and could predict its development. Bone Marrow Transplant. 2017;52(9):1317-25.
59. Sano H, Hilinski JA, Qayed M, Applegate K, Newton JG, Watkins B, et al. Early blood stream infection following allogeneic hematopoietic stem cell transplantation is a risk factor for acute grade III-IV GVHD in children and adolescents. Pediatr Blood Cancer. 2017.
60. Cario E. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. Gut. 2005;54(8):1182-93.
61. Kawachi S, Jennings S, Panes J, Cockrell A, Laroux FS, Gray L, et al. Cytokine and endothelial cell adhesion molecule expression in interleukin-10-deficient mice. Am J Physiol Gastrointest Liver Physiol. 2000;278(5):G734-43.
62. Piguet PF, Vesin C, Guo J, Donati Y, Barazzone C. TNF-induced enterocyte apoptosis in mice is mediated by the TNF receptor 1 and does not require p53. Eur J Immunol. 1998;28(11):3499-505.
63. Hill GR, Koyama M. Cytokines and Co-stimulation in Acute Graft-versus-Host Disease. Blood. 2020.

64. Kägi D, Vignaux F, Ledermann B, Bürki K, Depraetere V, Nagata S, et al. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science. 1994;265(5171):528-30.
65. Vakkila J, Hurme M. Both dendritic cells and monocytes induce autologous and allogeneic T cells receptive to interleukin 2. Scand J Immunol. 1990;31(1):75-83.
66. Nestel FP, Price KS, Seemayer TA, Lapp WS. Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. J Exp Med. 1992;175(2):405-13.
67. van Bekkum DW, Roodenburg J, Heidt PJ, van der Waaij D. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. J Natl Cancer Inst. 1974;52(2):401-4.
68. Vossen JM, Guiot HF, Lankester AC, Vossen AC, Bredius RG, Wolterbeek R, et al. Complete suppression of the gut microbiome prevents acute graft-versus-host disease following allogeneic bone marrow transplantation. PLoS One. 2014;9(9):e105706.
69. Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant. 2014;20(5):640-5.
70. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood. 2014;124(7):1174-82.
71. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal Blautia Is Associated with Reduced Death from Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2015;21(8):1373-83.
72. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012;55(7):905-14.
73. Romick-Rosendale LE, Haslam DB, Lane A, Denson L, Lake K, Wilkey A, et al. Antibiotic Exposure and Reduced Short Chain Fatty Acid Production after Hematopoietic Stem Cell Transplant. Biol Blood Marrow Transplant. 2018.
74. Levine JE, Huber E, Hammer ST, Harris AC, Greenson JK, Braun TM, et al. Low Paneth cell numbers at onset of gastrointestinal graft-versus-host disease identify patients at high risk for nonrelapse mortality. Blood. 2013;122(8):1505-9.
75. Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uryu H, et al. Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of alpha-defensins. Blood. 2012;120(1):223-31.
76. Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. Nat Immunol. 2016;17(5):505-13.
77. Di Ianni M, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood. 2011;117(14):3921-8.
78. Miura Y, Thoburn CJ, Bright EC, Phelps ML, Shin T, Matsui EC, et al. Association of Foxp3 regulatory gene expression with graft-versus-host disease. Blood. 2004;104(7):2187-93.
79. Rieger K, Loddenkemper C, Maul J, Fietz T, Wolff D, Terpe H, et al. Mucosal FOXP3+ regulatory T cells are numerically deficient in acute and chronic GvHD. Blood. 2006;107(4):1717-23.
80. Holler E, Greinix H, Zeiser R. Acute Graft-Versus-Host Disease. In: Carreras E, Dufour C, Mohty M, Kröger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. Cham (CH): Springer

Copyright 2019, EBMT and the Author(s). 2019. p. 323-30.
81. Ibrahim RB, Abidi MH, Cronin SM, Lum LG, Al-Kadhimi Z, Ratanatharathorn V, et al. Nonabsorbable corticosteroids use in the treatment of gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant. 2009;15(4):395-405.
82. Weisdorf DJ, Snover DC, Haake R, Miller WJ, McGlave PB, Blazar B, et al. Acute upper gastrointestinal graft-versus-host disease: clinical significance and response to immunosuppressive therapy. Blood. 1990;76(3):624-9.
83. Mehta RS, Cao Q, Holtan S, MacMillan ML, Weisdorf DJ. Upper GI GVHD: similar outcomes to other grade II graft-versus-host disease. Bone Marrow Transplant. 2017;52(8):1180-6.
84. MacMillan ML, Holtan SG, Rashidi A, DeFor TE, Blazar BR, Weisdorf DJ. Pediatric acute GVHD: clinical phenotype and response to upfront steroids. Bone Marrow Transplant. 2019.
85. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. Leukemia. 2015;29(10):2062-8.
86. Parikh SH, Satwani P, Ahn KW, Sahr NA, Fretham C, Abraham AA, et al. Survival Trends in Infants Undergoing Allogeneic Hematopoietic Cell Transplant. JAMA Pediatr. 2019;173(5):e190081.
87. Schmidt-Hieber M, Tridello G, Ljungman P, Mikulska M, Knelange N, Blaise D, et al. The prognostic impact of the cytomegalovirus serostatus in patients with chronic hematological malignancies after allogeneic hematopoietic stem cell transplantation: a report from the Infectious Diseases Working Party of EBMT. Ann Hematol. 2019;98(7):1755-63.
88. Shulman HM, Kleiner D, Lee SJ, Morton T, Pavletic SZ, Farmer E, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. Biol Blood Marrow Transplant. 2006;12(1):31-47.
89. Salamonowicz M, Ociepa T, Fraczkiewicz J, Szmydki-Baran A, Matysiak M, Czyzewski K, et al. Incidence, course, and outcome of Clostridium difficile infection in children with hematological malignancies or undergoing hematopoietic stem cell transplantation. Eur J Clin Microbiol Infect Dis. 2018;37(9):1805-12.
90. Flerlage T, Hayden R, Cross SJ, Dallas R, Srinivasan A, Tang L, et al. Rotavirus Infection in Pediatric Allogeneic Hematopoietic Cell Transplant Recipients: Clinical Course and Experience Using Nitazoxanide and Enterally Administered Immunoglobulins. Pediatr Infect Dis J. 2018;37(2):176-81.
91. Ye X, Van JN, Munoz FM, Revell PA, Kozinetz CA, Krance RA, et al. Noroviruses as a Cause of Diarrhea in Immunocompromised Pediatric Hematopoietic Stem Cell and Solid Organ Transplant Recipients. Am J Transplant. 2015;15(7):1874-81.
92. Srinivasan A, Klepper C, Sunkara A, Kang G, Carr J, Gu Z, et al. Impact of adenoviral stool load on adenoviremia in pediatric hematopoietic stem cell transplant recipients. Pediatr Infect Dis J. 2015;34(6):562-5.
93. Ljungman P, Perez-Bercoff L, Jonsson J, Avetisyan G, Sparrelid E, Aschan J, et al. Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. Haematologica. 2006;91(1):78-83.
94. Kamboj M, Mihu CN, Sepkowitz K, Kernan NA, Papanicolaou GA. Work-up for infectious diarrhea after allogeneic hematopoietic stem cell transplantation: single specimen testing results in cost savings without compromising diagnostic yield. Transpl Infect Dis. 2007;9(4):265-9.

95. McFarland LV. Renewed interest in a difficult disease: Clostridium difficile infections--epidemiology and current treatment strategies. Curr Opin Gastroenterol. 2009;25(1):24-35.
96. Brook I. Pseudomembranous colitis in children. J Gastroenterol Hepatol. 2005;20(2):182-6.
97. Nomura K, Fujimoto Y, Yamashita M, Morimoto Y, Ohshiro M, Sato K, et al. Absence of pseudomembranes in Clostridium difficile-associated diarrhea in patients using immunosuppression agents. Scand J Gastroenterol. 2009;44(1):74-8.
98. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363(22):2091-101.
99. Jaing TH, Chang TY, Chen SH, Wen YC, Yu TJ, Lee CF, et al. Factors associated with cytomegalovirus infection in children undergoing allogeneic hematopoietic stem-cell transplantation. Medicine (Baltimore). 2019;98(4):e14172.
100. Al Yazidi LS, Mitchell R, Palasanthiran P, O'Brien TA, McMullan B. Management and prevention of cytomegalovirus infection in paediatric hematopoietic stem cell transplant (HSCT) recipients: A binational survey. Pediatr Transplant. 2019;23(5):e13458.
101. Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood. 2009;113(23):5711-9.
102. Uhlin M, Wikell H, Sundin M, Blennow O, Maeurer M, Ringden O, et al. Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation. Haematologica. 2014;99(2):346-52.
103. Rouce RH, Louis CU, Heslop HE. Epstein-Barr virus lymphoproliferative disease after hematopoietic stem cell transplant. Curr Opin Hematol. 2014;21(6):476-81.
104. Llaurador G, McLaughlin L, Wistinghausen B. Management of post-transplant lymphoproliferative disorders. Curr Opin Pediatr. 2017;29(1):34-40.
105. García-Cadenas I, Yáñez L, Jarque I, Martino R, Pérez-Simón JA, Valcárcel D, et al. Frequency, characteristics, and outcome of PTLD after allo-SCT: A multicenter study from the Spanish group of blood and marrow transplantation (GETH). Eur J Haematol. 2019;102(6):465-71.
106. Parker A, Bowles K, Bradley JA, Emery V, Featherstone C, Gupte G, et al. Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients - BCSH and BTS Guidelines. Br J Haematol. 2010;149(5):675-92.
107. Westerhoff M, Lamps LW. Mucosal Biopsy After Bone Marrow Transplantation. Surg Pathol Clin. 2017;10(4):909-30.
108. van Montfrans J, Schulz L, Versluys B, de Wildt A, Wolfs T, Bierings M, et al. Viral PCR positivity in stool before allogeneic hematopoietic cell transplantation is strongly associated with acute intestinal graft-versus-host disease. Biol Blood Marrow Transplant. 2015;21(4):772-4.
109. Bhutani D, Jaiyeoba C, Kim S, Naylor P, Uberti JP, Ratanatharathorn V, et al. Relationship between clostridium difficile infection and gastrointestinal graft versus host disease in recipients of allogeneic stem cell transplantation. Bone Marrow Transplant. 2018.
110. Gonzales F, Bruno B, Alarcon Fuentes M, De Berranger E, Guimber D, Behal H, et al. Better early outcome with enteral rather than parenteral nutrition in children undergoing MAC allo-SCT. Clin Nutr. 2017.
111. Yang H, Feng Y, Sun X, Teitelbaum DH. Enteral versus parenteral nutrition: effect on intestinal barrier function. Ann N Y Acad Sci. 2009;1165:338-46.
112. Wildhaber BE, Lynn KN, Yang H, Teitelbaum DH. Total parenteral nutrition-induced apoptosis in mouse intestinal epithelium: regulation by the Bcl-2 protein family. Pediatr Surg Int. 2002;18(7):570-5.

113. Locatelli F, Pession A, Bonetti F, Maserati E, Prete L, Pedrazzoli P, et al. Busulfan, cyclophosphamide and melphalan as conditioning regimen for bone marrow transplantation in children with myelodysplastic syndromes. Leukemia. 1994;8(5):844-9.
114. Hassan M, Ljungman P, Bolme P, Ringdén O, Syrůcková Z, Békàssy A, et al. Busulfan bioavailability. Blood. 1994;84(7):2144-50.
115. Hassan M. The role of busulfan in bone marrow transplantation. Med Oncol. 1999;16(3):166-76.
116. Cheuk DK, Wang P, Lee TL, Chiang AK, Ha SY, Lau YL, et al. Risk factors and mortality predictors of hepatic veno-occlusive disease after pediatric hematopoietic stem cell transplantation. Bone Marrow Transplant. 2007;40(10):935-44.
117. Tsuboi K, Kishi K, Ohmachi K, Yasuda Y, Shimizu T, Inoue H, et al. Multivariate analysis of risk factors for hemorrhagic cystitis after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2003;32(9):903-7.
118. Nevill TJ, Barnett MJ, Klingemann HG, Reece DE, Shepherd JD, Phillips GL. Regimen-related toxicity of a busulfan-cyclophosphamide conditioning regimen in 70 patients undergoing allogeneic bone marrow transplantation. J Clin Oncol. 1991;9(7):1224-32.
119. Lucchini G, Labopin M, Beohou E, Dalissier A, Dalle JH, Cornish J, et al. Impact of Conditioning Regimen on Outcomes for Children with Acute Myeloid Leukemia Undergoing Transplantation in First Complete Remission. An Analysis on Behalf of the Pediatric Disease Working Party of the European Group for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2017;23(3):467-74.
120. Lightdale JR, Acosta R, Shergill AK, Chandrasekhara V, Chathadi K, Early D, et al. Modifications in endoscopic practice for pediatric patients. Gastrointest Endosc. 2014;79(5):699-710.
121. Thomson M, Tringali A, Dumonceau JM, Tavares M, Tabbers MM, Furlano R, et al. Paediatric Gastrointestinal Endoscopy: European Society for Paediatric Gastroenterology Hepatology and Nutrition and European Society of Gastrointestinal Endoscopy Guidelines. J Pediatr Gastroenterol Nutr. 2017;64(1):133-53.
122. Dignan FL, Clark A, Amrolia P, Cornish J, Jackson G, Mahendra P, et al. Diagnosis and management of acute graft-versus-host disease. Br J Haematol. 2012;158(1):30-45.
123. Brodoefel H, Bethge W, Vogel M, Fenchel M, Faul C, Wehrmann M, et al. Early and late-onset acute GvHD following hematopoietic cell transplantation: CT features of gastrointestinal involvement with clinical and pathological correlation. Eur J Radiol. 2010;73(3):594-600.
124. Budjan J, Michaely HJ, Attenberger U, Haneder S, Heidenreich D, Kreil S, et al. Assessment of acute intestinal graft versus host disease by abdominal magnetic resonance imaging at 3 Tesla. Eur Radiol. 2014;24(8):1835-44.
125. Gorg C, Wollenberg B, Beyer J, Stolte MS, Neubauer A. High-resolution ultrasonography in gastrointestinal graft-versus-host disease. Ann Hematol. 2005;84(1):33-9.
126. Ferrara JL, Harris AC, Greenson JK, Braun TM, Holler E, Teshima T, et al. Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. Blood. 2011;118(25):6702-8.
127. Lorenz F, Marklund S, Werner M, Palmqvist R, Wahlin BE, Wahlin A. Fecal calprotectin as a biomarker of intestinal graft versus host disease after allogeneic hematopoietic stem cell transplantation. Sci Rep. 2015;5:7920.
128. O'Meara A, Kapel N, Xhaard A, Sicre de Fontbrune F, Manéné D, Dhedin N, et al. Fecal calprotectin and α1-antitrypsin dynamics in gastrointestinal GvHD. Bone Marrow Transplant. 2015;50(8):1105-9.

129. Malik MN, Rafae A, Durer C, Durer S, Anwer F. Fecal Calprotectin as a Diagnostic and Prognostic Biomarker for Gastrointestinal Graft Versus Host Disease: A Systematic Review of Literature. Cureus. 2019;11(2):e4143.
130. Sultan M, Ramprasad J, Jensen MK, Margolis D, Werlin S. Endoscopic diagnosis of pediatric acute gastrointestinal graft-versus-host disease. J Pediatr Gastroenterol Nutr. 2012;55(4):417-20.
131. Thompson B, Salzman D, Steinhauer J, Lazenby AJ, Wilcox CM. Prospective endoscopic evaluation for gastrointestinal graft-versus-host disease: determination of the best diagnostic approach. Bone Marrow Transplant. 2006;38(5):371-6.
132. Kreft A, Mottok A, Mesteri I, Cardona DM, Janin A, Kuhl AA, et al. Consensus diagnostic histopathological criteria for acute gastrointestinal graft versus host disease improve interobserver reproducibility. Virchows Arch. 2015;467(3):255-63.
133. Washington K, Jagasia M. Pathology of graft-versus-host disease in the gastrointestinal tract. Hum Pathol. 2009;40(7):909-17.
134. Cardona DM, Detweiler CJ, Shealy MJ, Sung AD, Wild DM, Poleski MH, et al. Use of the National Institutes of Health Consensus Guidelines Improves the Diagnostic Sensitivity of Gastrointestinal Graft-Versus-Host Disease. Arch Pathol Lab Med. 2018;142(9):1098-105.
135. Sauvestre F, Belleannee G, Breal C, Mohr C, Fong HI, Cossin S, et al. Histologic analysis has a prognostical value in colorectal biopsies assessed for suspicion of graft-versus-host disease. Virchows Arch. 2018;472(2):213-20.
136. Gomez AJ, Arai S, Higgins JP, Kambham N. Clinicopathologic Threshold of Acute Colorectal Graft-versus-Host Disease. Arch Pathol Lab Med. 2016;140(6):570-7.
137. Lin J, Fan R, Zhao Z, Cummings OW, Chen S. Is the presence of 6 or fewer crypt apoptotic bodies sufficient for diagnosis of graft versus host disease? A decade of experience at a single institution. Am J Surg Pathol. 2013;37(4):539-47.
138. Im JS, Abraham SC, Saliba RM, Rondon G, Ross WA, Rashid A, et al. Histologic Grade 1 Is Associated With Increased Nonrelapsed Mortality in Lower Gastrointestinal Graft Versus Host Disease. Am J Surg Pathol. 2017;41(11):1483-90.
139. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HL-A-matched sibling donors. Transplant Proc. 1974;6(4):367-71.
140. Melson J, Jakate S, Fung H, Arai S, Keshavarzian A. Crypt loss is a marker of clinical severity of acute gastrointestinal graft-versus-host disease. Am J Hematol. 2007;82(10):881-6.
141. Nazer H, Walker-Smith JA, Davidson K, Williams CB. Outpatient paediatric fibreoptic proctosigmoidoscopy: possible and useful. Br Med J (Clin Res Ed). 1983;286(6362):352.
142. Tobias JD. Applications of nitrous oxide for procedural sedation in the pediatric population. Pediatr Emerg Care. 2013;29(2):245-65.
143. Michaud L, Gottrand F, Ganga-Zandzou PS, Ouali M, Vetter-Laffargue A, Lambilliotte A, et al. Nitrous oxide sedation in pediatric patients undergoing gastrointestinal endoscopy. J Pediatr Gastroenterol Nutr. 1999;28(3):310-4.
144. Lee KJ, Choi SJ, Yang HR, Chang JY, Kang HJ, Shin HY, et al. Stepwise Endoscopy Based on Sigmoidoscopy in Evaluating Pediatric Graft-versus-Host Disease. Pediatr Gastroenterol Hepatol Nutr. 2016;19(1):29-37.
145. Nydegger A, Catto-Smith AG, Tiedemann K, Hardikar W. Diagnosis of gastrointestinal graft-versus-host disease--is rectal biopsy enough? Pediatr Blood Cancer. 2007;48(5):561-6.
146. Crowell KR, Patel RA, Fluchel M, Lowichik A, Bryson S, Pohl JF. Endoscopy in the diagnosis of intestinal graft-versus-host disease: is lower endoscopy with

biopsy as effective in diagnosis as upper endoscopy combined with lower endoscopy? Pediatr Blood Cancer. 2013;60(11):1798-800.
147. Karamchandani DM, Chetty R. Apoptotic colopathy: a pragmatic approach to diagnosis. J Clin Pathol. 2018;71(12):1033-40.
148. Navarre WW, Zychlinsky A. Pathogen-induced apoptosis of macrophages: a common end for different pathogenic strategies. Cell Microbiol. 2000;2(4):265-73.
149. Bucker R, Krug SM, Moos V, Bojarski C, Schweiger MR, Kerick M, et al. Campylobacter jejuni impairs sodium transport and epithelial barrier function via cytokine release in human colon. Mucosal Immunol. 2018;11(2):474-85.
150. Troeger H, Loddenkemper C, Schneider T, Schreier E, Epple HJ, Zeitz M, et al. Structural and functional changes of the duodenum in human norovirus infection. Gut. 2009;58(8):1070-7.
151. Chaibi C, Cotte-Laffitte J, Sandre C, Esclatine A, Servin AL, Quero AM, et al. Rotavirus induces apoptosis in fully differentiated human intestinal Caco-2 cells. Virology. 2005;332(2):480-90.
152. Chumbler NM, Farrow MA, Lapierre LA, Franklin JL, Lacy DB. Clostridium difficile Toxins TcdA and TcdB Cause Colonic Tissue Damage by Distinct Mechanisms. Infect Immun. 2016;84(10):2871-7.
153. Moss SF, Sordillo EM, Abdalla AM, Makarov V, Hanzely Z, Perez-Perez GI, et al. Increased gastric epithelial cell apoptosis associated with colonization with cagA + Helicobacter pylori strains. Cancer Res. 2001;61(4):1406-11.
154. Epstein RJ, McDonald GB, Sale GE, Shulman HM, Thomas ED. The diagnostic accuracy of the rectal biopsy in acute graft-versus-host disease: a prospective study of thirteen patients. Gastroenterology. 1980;78(4):764-71.
155. Jalili-Firoozinezhad S, Prantil-Baun R, Jiang A, Potla R, Mammoto T, Weaver JC, et al. Modeling radiation injury-induced cell death and countermeasure drug responses in a human Gut-on-a-Chip. Cell Death Dis. 2018;9(2):223.
156. Nguyen T, Park JY, Scudiere JR, Montgomery E. Mycophenolic acid (cellcept and myofortic) induced injury of the upper GI tract. Am J Surg Pathol. 2009;33(9):1355-63.
157. Papadimitriou JC, Cangro CB, Lustberg A, Khaled A, Nogueira J, Wiland A, et al. Histologic features of mycophenolate mofetil-related colitis: a graft-versus-host disease-like pattern. Int J Surg Pathol. 2003;11(4):295-302.
158. Yoshino T, Nakase H, Honzawa Y, Matsumura K, Yamamoto S, Takeda Y, et al. Immunosuppressive effects of tacrolimus on macrophages ameliorate experimental colitis. Inflamm Bowel Dis. 2010;16(12):2022-33.
159. Fujino M, Kim Y, Ito M. Intestinal thrombotic microangiopathy induced by FK506 in rats. Bone Marrow Transplant. 2007;39(6):367-72.
160. Cao BH, Mortensen K, Tornehave D, Larsson LI. Apoptosis in rat gastric antrum: Evidence that regulation by food intake depends on nitric oxide synthase. J Histochem Cytochem. 2000;48(1):123-31.
161. Parfitt JR, Driman DK. Pathological effects of drugs on the gastrointestinal tract: a review. Hum Pathol. 2007;38(4):527-36.
162. Welch DC, Wirth PS, Goldenring JR, Ness E, Jagasia M, Washington K. Gastric graft-versus-host disease revisited: does proton pump inhibitor therapy affect endoscopic gastric biopsy interpretation? Am J Surg Pathol. 2006;30(4):444-9.
163. European Medicines Agency. GUIDELINE ON CLINICAL EVALUATION OF DIAGNOSTIC AGENTS. 2009.:https://www.ema.europa.eu/en/clinical-evaluation-diagnostic-agents.
164. Banerjee P, Rossi MG, Anghelescu DL, Liu W, Breazeale AM, Reddick WE, et al. Association Between Anesthesia Exposure and Neurocognitive and Neuroimaging

Outcomes in Long-term Survivors of Childhood Acute Lymphoblastic Leukemia. JAMA Oncol. 2019.
165. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology. 2009;110(4):796-804.
166. O'Leary JD, Warner DO. What do recent human studies tell us about the association between anaesthesia in young children and neurodevelopmental outcomes? Br J Anaesth. 2017;119(3):458-64.
167. Sierra A, Ecochard-Dugelay E, Bellaiche M, Tilea B, Cave H, Viala J. Biopsy-Induced Duodenal Hematoma Is Not an Infrequent Complication Favored by Bone Marrow Transplantation. J Pediatr Gastroenterol Nutr. 2016;63(6):627-32.
168. Khan K, Schwarzenberg SJ, Sharp H, Jessurun J, Gulbahce HE, Defor T, et al. Diagnostic endoscopy in children after hematopoietic stem cell transplantation. Gastrointest Endosc. 2006;64(3):379-85; quiz 89-92.
169. Matsumura-Kimoto Y, Inamoto Y, Tajima K, Kawajiri A, Tanaka T, Hirakawa T, et al. Association of Cumulative Steroid Dose with Risk of Infection after Treatment for Severe Acute Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2016;22(6):1102-7.
170. Fisher BT, Danziger-Isakov L, Sweet LR, Munoz FM, Maron G, Tuomanen E, et al. A Multicenter Consortium to Define the Epidemiology and Outcomes of Inpatient Respiratory Viral Infections in Pediatric Hematopoietic Stem Cell Transplant Recipients. J Pediatric Infect Dis Soc. 2018;7(4):275-82.
171. Majhail NS, Challa TR, Mulrooney DA, Baker KS, Burns LJ. Hypertension and diabetes mellitus in adult and pediatric survivors of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2009;15(9):1100-7.
172. Wolff D, Ayuk F, Elmaagacli A, Bertz H, Lawitschka A, Schleuning M, et al. Current practice in diagnosis and treatment of acute graft-versus-host disease: results from a survey among German-Austrian-Swiss hematopoietic stem cell transplant centers. Biol Blood Marrow Transplant. 2013;19(5):767-76.
173. Gassas A, Krueger J, Schechter T, Zaidman I, Asim M, Ali M. Safety and Role of Gastrointestinal Endoscopy in the Management of Gastrointestinal Acute GVHD in Children After Hematopoietic Stem Cell Transplantation. J Pediatr Hematol Oncol. 2016;38(6):453-6.
174. Niemeyer CM, Flotho C. Juvenile myelomonocytic leukemia: who's the driver at the wheel? Blood. 2019;133(10):1060-70.
175. Locatelli F, Strahm B. How I treat myelodysplastic syndromes of childhood. Blood. 2018;131(13):1406-14.
176. Hassan Z. Management of refractory hemorrhagic cystitis following hematopoietic stem cell transplantation in children. Pediatr Transplant. 2011;15(4):348-61.
177. Jones RJ, Lee KS, Beschorner WE, Vogel VG, Grochow LB, Braine HG, et al. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation. 1987;44(6):778-83.
178. Samuels BL, Bitran JD. High-dose intravenous melphalan: a review. J Clin Oncol. 1995;13(7):1786-99.
179. Goldberg J, Jacobsohn DA, Zahurak ML, Vogelsang GB. Gastrointestinal toxicity from the preparative regimen is associated with an increased risk of graft-versus-host disease. Biol Blood Marrow Transplant. 2005;11(2):101-7.
180. Fallows G, Rubinger M, Bernstein CN. Does gastroenterology consultation change management of patients receiving hematopoietic stem cell transplantation? Bone Marrow Transplant. 2001;28(3):289-94.

181. Castilla-Llorente C, Martin PJ, McDonald GB, Storer BE, Appelbaum FR, Deeg HJ, et al. Prognostic factors and outcomes of severe gastrointestinal GVHD after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2014;49(7):966-71.
182. Nevo S, Enger C, Swan V, Wojno KJ, Fuller AK, Altomonte V, et al. Acute bleeding after allogeneic bone marrow transplantation: association with graft versus host disease and effect on survival. Transplantation. 1999;67(5):681-9.
183. Gatza E, Reddy P, Choi SW. Prevention and Treatment of Acute Graft-versus-Host Disease in Children, Adolescents, and Young Adults. Biol Blood Marrow Transplant. 2020;26(5):e101-e12.
184. Nikiforow S, Wang T, Hemmer M, Spellman S, Akpek G, Antin JH, et al. Upper gastrointestinal acute graft-versus-host disease adds minimal prognostic value in isolation or with other graft-versus-host disease symptoms as currently diagnosed and treated. Haematologica. 2018;103(10):1708-19.
185. Beckerson J, Szydlo RM, Hickson M, Mactier CE, Innes AJ, Gabriel IH, et al. Impact of route and adequacy of nutritional intake on outcomes of allogeneic haematopoietic cell transplantation for haematologic malignancies. Clin Nutr. 2019;38(2):738-44.
186. Zheng HB, Yeung C, Summers C, Lee D, Wahbeh G, Braly K, et al. Dietary Therapy in Conjunction With Immunosuppression to Treat Gastrointestinal Graft-versus-host Disease (GVHD). J Pediatr Gastroenterol Nutr. 2019;69(1):e20-e2.
187. Borrelli O, Cordischi L, Cirulli M, Paganelli M, Labalestra V, Uccini S, et al. Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn's disease: a randomized controlled open-label trial. Clin Gastroenterol Hepatol. 2006;4(6):744-53.
188. Biagi E, Zama D, Nastasi C, Consolandi C, Fiori J, Rampelli S, et al. Gut microbiota trajectory in pediatric patients undergoing hematopoietic SCT. Bone Marrow Transplant. 2015;50(7):992-8.
189. Ladas EJ, Bhatia M, Chen L, Sandler E, Petrovic A, Berman DM, et al. The safety and feasibility of probiotics in children and adolescents undergoing hematopoietic cell transplantation. Bone Marrow Transplant. 2016;51(2):262-6.
190. Schoemans HM, Goris K, Van Durm R, Vanbrabant K, De Geest S, Maertens J, et al. Accuracy and usability of the eGVHD app in assessing the severity of graft-versus-host disease at the 2017 EBMT annual congress. Bone Marrow Transplant. 2018;53(4):490-4.