AUS DEM LEHRSTUHL
FÜR INNERE MEDIZIN III
PROF. DR. WOLFGANG HERR
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

PROGNOSTIC SIGNIFICANCE OF SCREENING GASTROINTESTINAL BIOPSIES, REG3ALPHA LEVELS AND EASIX-SCORE FOR DEVELOPMENT OF GASTROINTESTINAL GVHD AND NON-RELAPSE-MORTALITY IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION – A RETROSPECTIVE ANALYSIS

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

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Tag der mündlichen Prüfung: 09.08.2022

Zusammenfassung

Die akute Transplantat-gegen-Wirt-Reaktion (*Graft-versus-Host-Disease, GVHD*) ist nach wie vor die schwerwiegendste Komplikation der allogenen Stammzelltransplantation. Wenn sie trotz Prophylaxe auftritt, wird sie derzeit noch einheitlich mit hochdosierten Steroiden behandelt. Versagen die Steroide, so ist die Prognose der GVHD ungünstig, vor allem bei gastrointestinaler Beteiligung. Mehr als 80% der Patienten mit steroidrefraktärer GVHD versterben an dieser Erkrankung.

Aufgrund der insgesamt schlechten Prognose, ist die Individualisierung des therapeutischen Ansatzes erforderlich. Die Identifizierung von Patienten mit hohem Risiko eine schwere akute GVHD zu entwickeln, könnte eine risikoadaptierte, individuelle Primärtherapie oder sogar eine präemptive Therapie ermöglichen.

Seit 2005 werden am Klinikum der Universität Regensburg gastrointestinale Frühbiopsien im Rahmen eines Screening-Programms durchgeführt. Diese gastrointestinalen Frühbiopsien erfolgten bei Patienten, die keine Symptome einer akuten GVHD zeigten und auch zur Abklärung von gastrointestinalen Symptomen, die die klinischen Kriterien für die Diagnose einer GVHD nicht erfüllten. In der Zwischenauswertung zeigte sich, dass in bis zu einem Viertel dieser Frühbiopsien bereits erhebliche Apoptosen vorhanden waren. In dieser Dissertation wurden retrospektiv die histologischen Ergebnisse aller gastrointestinaler Frühbiopsien gesammelt und auf ihre prognostische Bedeutung (Ausmaß der späteren GVHD, transplantationsassozierte Mortalität) analysiert. Gleichzeitig wurden weitere prognostische Parameter einer akuten GVHD (Serum Reg3alfa und EASIX Luft-Score) ergänzt. Die prognostische Bedeutung dieser Werte wurde in gleicher Weise isoliert berechnet und dann mit der der Frühbiopsien verglichen.

Contents

1. Introduction

1.1 The secondary syndrome

In his paper, published in 1964 in the British Medical Journal (1), Georg Mathé, one of the pioneers of the allogeneic hematopoietic stem-cell transplantation (HSCT), reports on practical problems and difficulties encountered in his clinical practice. He focuses on the "secondary syndrome" – today better known as the graft-versus-host disease (GVHD) – and reports on his experiences with it, not only in humans but also in various experimental animal species. He describes in detail the clinical features of the secondary syndrome – its biochemical, hematologic, and histological characteristics. Mathé concludes that "because it is particularly severe in man," the secondary syndrome "constitutes the principal obstacle to the routine use of allogeneic haemopoietic cell grafts." Despite massive progress in our understanding of its pathophysiology, and continual improvements in the treatment, GVHD (or the secondary syndrome in the terminology of the 1960s) remains the most important limitation of the allogeneic HSCT.

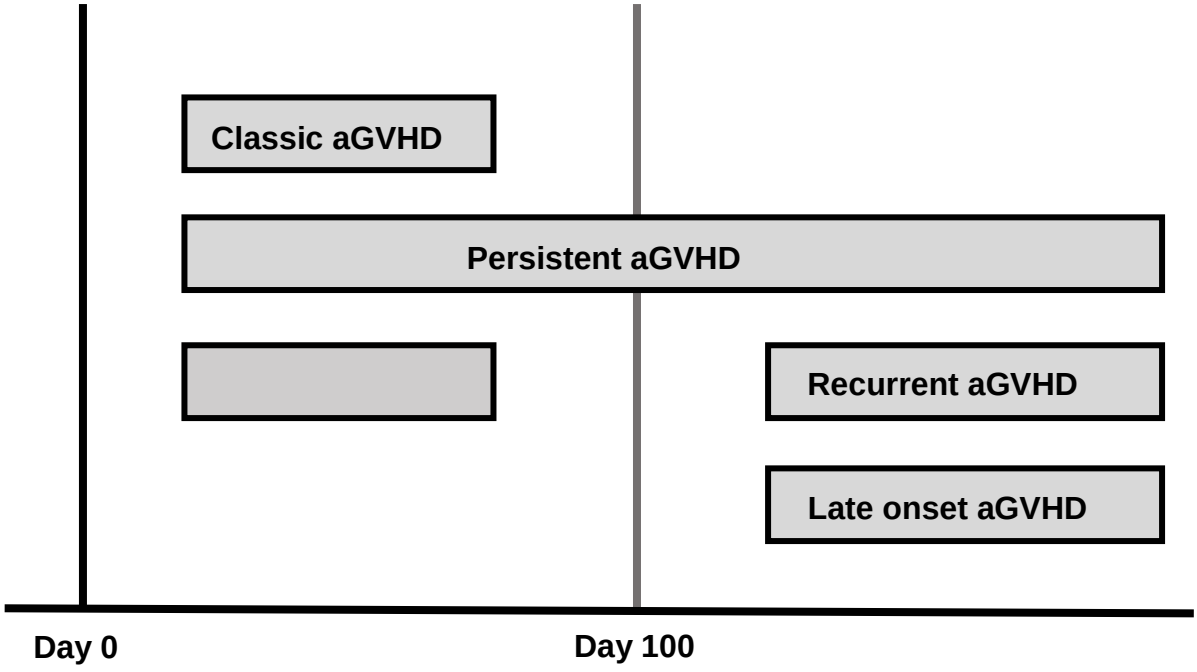
1.2. Graft-versus-host disease (GVHD)

Graft-versus-host disease (GVHD) is a clinical syndrome caused by the response of transplanted donor allogeneic cells to the histocompatibility antigens expressed on tissues of the transplantation recipient. It remains one of the leading causes of morbidity and mortality in the post-allogeneic-HSCT setting. GVHD is divided into two separate entities – *acute* graft-versus-host disease (aGVHD) and *chronic* graft-versus-host disease (cGVHD), each with distinct clinical and histological presentations.

The traditional, temporal distinction between these two entities (whereby aGVHD occurs within the first one hundred days after HSCT, while cGVHD takes place after this threshold) is now mostly seen as outdated. Diagnosis and treatment of the acute and chronic forms of the disease are based on their well-defined clinical and histological characteristics, which are independent of the symptoms' moment of manifestation (2). Late onset of aGVHD is uncommon *but is very well documented, for example in patients who have received reduced-intensity conditioning chemotherapy or additional donor lymphocyte infusion.* The current classification of aGVHD, which depends on the time of its manifestation, is depicted in Graph 1.

Graph 1

Classification of Acute GVHD According to Time of its Onset



According to the National Institute of Health criteria, chronic GVHD with its defining characteristics and features can occur at any time after allogeneic HSCT (2–4). Both acute and chronic forms of GVHD may also appear simultaneously and this condition is named “overlap syndrome” (2–4).

In the next session, I will focus on the acute GVHD, with an emphasis on the acute GVHD of gastrointestinal tract.

1.3. Acute GVHD

Acute GVHD is an inflammatory allogeneic response in the skin, the liver, and the gastrointestinal tract that develops in approximately 35–50% of hematopoietic stem-cell transplant recipients (5). Acute GVHD can occur independently in any of its three target organs, or can affect more of them simultaneously. The usual clinical findings are inflammatory maculopapular erythematous skin rash, jaundice, when the liver is affected, and various gastrointestinal manifestations such as anorexia, nausea, vomiting, secretory diarrhea sometimes associated with severe pain and bleeding.

Acute GVHD occurs as a result of dysregulated cell-mediated immunity, when the activation of proinflammatory cytokine cascades and donor alloreactive T cells causes the destruction of healthy recipient tissues (6–8). Pathophysiological changes in aGVHD can be divided into three phases (6,7,9–11). Initially, as a part of the first phase, the tissue damage occurs. It happens as a result of the underlying disease itself or of its earlier treatment, of concomitant infections or due to the preparative conditioning chemo- or radiotherapy. This tissue damage causes the release of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and induces the secretion of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) (12) and interleukin 1 (IL-1) (6,7,11). In the second phase of aGVHD development released cytokines lead to the activation of hosts antigen-presenting cells (APCs) (6,7,9,10). Activated APCs interact with donor T cells which then causes their activation, proliferation, differentiation and migration. Activated donor T cells secrete inflammatory cytokines, which in turn stimulate production of inflammatory chemokines, thus recruiting effector cells into target organs. In the third phase of acute GVHD this complex cascade of multiple effectors leads to the destruction of the healthy host tissue through both specific and non-specific mechanisms. This amplification of the local tissue injury further promotes the inflammatory response and establishes a positive inflammatory feedback loop (6,10). The destruction of target normal tissues leads to classical symptoms and signs of acute GVHD.

The symptoms and signs of acute GVHD are routinely graded for their severity in everyday clinical practice. One of the first widely adopted classification systems was published in 1974 by Glucksberg (13). Glucksberg introduced the model of grading the involvement of three affected organs (skin, liver, gastrointestinal tract) separately, on a scale from 0 to 4. These three scores are then combined for an overall grade of mild (Grade I), moderate (Grade II), severe (Grade III) or life-threatening GVHD (Grade IV). This scoring system was modified in 1994 at the Consensus Conference held in Keystone (14). Most recently, the Mount Sinai Acute GVHD International Consortium (MAGIC) has revisited these criteria, and recommended more precise definitions (4,15). MAGIC criteria are considered to be the most current and detailed for the diagnosis and severity grading of acute GVHD (4). MAGIC guidelines for assessment of severity of acute GVHD are shown in the Table 1 (15).

Table 1. Mount Sinai Acute GVHD International Consortium (MAGIC) Guidelines for Assessment of the Severity of Acute GVHD

Stage	Skin (active erythema)	Liver – bilirubin	Upper GI tract	Lower GI tract (stool output/day)
0	No active GVHD rash	< 2 mg/dL	No or intermittent nausea, vomiting, or anorexia	< 500 mL/day or < 3 episodes/day
1	Maculopapular rash < 25% BSA	2–3 mg/dL	Persistent nausea, vomiting or anorexia	500–999 mL/day or 3–4 episodes/day
2	Maculopapular rash 25 – 50% BSA	3.1–6 mg/dL	-	1000–1500 mL/day or 5–7 episodes/day
3	Maculopapular rash > 50% BSA	6.1–15 mg/dL	-	> 1500 mL/day or > 7 episodes/day
4	Maculopapular rash > 50% BSA plus bullous formation > 5% BSA	> 15 mg/dL	-	Severe abdominal pain with or without ileus, or grossly bloody stool

Abbreviations: GI, gastrointestinal; GVHD, Graft-versus-Host Disease; mg, milligram, mL, milliliter; dL, deciliter; BSA, body surface area

Table 1. Mount Sinai Acute GVHD International Consortium (MAGIC) Guidelines for Assessment of the Severity of Acute GVHD – Continuation

Overall clinical grade (based upon most severe target-organ involvement):

Grade 0: No stage 1–4 of any organ

Grade I: Stage 1–2 skin without liver, upper GI, or lower GI involvement

Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI

Grade III: Stage 2–3 liver and/or stage 2–3 lower GI, with stage 0–3 skin and/or stage 0–1 upper GI

Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0–1 upper GI

1.4. Acute GVHD of the gastrointestinal (GI) tract

One of the primary target organs of acute GVHD is the gastrointestinal (GI) tract. Acute GVHD is often a pan-intestinal process, usually with differences in severity between the upper and the lower GI tract. Typical clinical manifestations are anorexia, nausea, vomiting, secretory diarrhea, which are sometimes accompanied by severe pain and bleeding. Patients with GI GVHD are rarely hungry or even suffer from severe anorexia. The diagnosis of acute gastrointestinal GVHD remains predominantly based on clinical findings after the exclusion of alternative causes (5). Clinical diagnosis should ideally be supported by positive histological findings, but obtaining a biopsy should not delay the initiation of treatment (5). Differential diagnoses include bacterial and viral gastroenteritis, pseudomembranous colitis, neutropenic colitis, drug-induced enteritis, or residual effects of conditioning chemo- or radiotherapy on gastrointestinal mucosa, which are often clinically indistinguishable from acute GVHD of the gut.

The most important pathophysiological changes in acute GVHD of the gut occur in the gastrointestinal mucosa, specifically in the mucosal crypts. Intestinal stem cells (ISCs) and Paneth cells, the key cellular targets of gastrointestinal acute GVHD, are located at the base of the mucosal crypts. Continuous antimicrobial protection of the intestinal stem-cell zone against pathogenic bacteria is of paramount importance for

maintaining the integrity of the mucosal barrier (16). This is achieved through various mechanisms. The epithelial surface is protected by a layer of mucus that provides a physical barrier against luminal pathogens. Antibodies of the secretory IgA class provide antigen-specific immune defense and prevent access of commensal and pathogenic microorganisms through the gastrointestinal epithelia. It has been estimated that up to three-quarters of the bacteria in the gut lumen are coated with secretory IgA (17). Secretory IgA antibodies, along with the wide range of antimicrobial peptides (AMPs) produced by Paneth cells, shape the composition of the gut microbiome and play a pivotal role in the modulation of its homeostasis (18).

The production of antimicrobial peptides is believed to prevent microbial invasion into the crypt microenvironment, thus providing protection for the intestinal stem-cell zone (16). An additional shielding effect at the gastrointestinal mucosal barrier is achieved through the function of the cells of the innate lymphoid system. These cells are found in abundance very close to the intestinal crypts, and their synthesis of various modulatory and antimicrobial peptides leads to a diverse and protective composition of the gut microbiome (18–20).

The initial damage to the gastrointestinal mucosa that leads to acute GVHD is unspecific. The toxic effects of conditioning chemo- or radiotherapy lead to apoptosis of enterocytes and to increased permeability of the epithelial barrier. The gut microbiome is heavily affected, and major changes in the distribution of bacteria occur in the post-conditioning neutropenic phase (20). Use of systemic antibiotics leads to the loss of the protective commensal bacteria (21,22) and to the overgrowth of pathogenic bacteria (23), with the predominance of enterococci (20). These changes in microbiome accelerate the already-existing injury of the gastrointestinal mucosal barrier. Mucosal damage and the consequent activation of antigen-presenting cells lead to the activation of donor T cells and the recruiting of effector cells (18). This complex cascade results in the further, immune-mediated, destruction of the epithelium of intestinal crypts, targeting especially the Paneth cells (24) and the intestinal stem cells (18).

Excessive activation of the local inflammatory response may not be the only pathophysiological change that occurs in acute GVHD of the gastrointestinal tract. As

suggested by Holler (19,25) a loss of intestinal immunoregulation seems to be a significant contributing factor. Furthermore, various defects in innate immunity signaling (such as single nucleotide polymorphisms of the innate immunity receptor *NOD2/CARD15)* are shown to influence the development of acute GVHD (25).

1.5. Treatment of gastrointestinal acute GVHD and its (in)efficacy

The use of systemic corticosteroids is a standard first-line therapy for gastrointestinal aGVHD (5). Daily use of two milligrams of methylprednisolone per kilogram of body weight is recommended as the starting dose (5,26). Increasing the dose of corticosteroids does not improve response rates and overall survival (27,28). The use of oral, non-absorbable corticosteroids may help to further reduce systemic dose requirements (29,30). Lower doses of corticosteroids may be adequate for isolated, acute GVHD of the upper gut. The chance of a response decreases with an increase in the grade of GVHD, but in general 40–50% of patients will respond to the treatment (31,32).

Failure to respond to the first-line treatment, an incomplete response, or GVHD recurrence after the initial dose reduction is common. Outcomes among steroid-refractory patients remain dismal despite continual developments and improved availability of second-line treatments (27,33). Extracorporeal photopheresis, anti–tumor-necrosis-factor-alpha antibodies, mammalian target of rapamycin (mTOR) inhibitors, mycophenolate mofetil and interleukin-2 receptor antibodies – all frequently used in the steroid-refractory setting (5,34) – rarely lead to complete and durable responses. A detailed review of treatment approaches to the steroid-refractory GVHD of the gut goes beyond the scope of this dissertation.

1.6. Role of histology in gastrointestinal aGVHD

The diagnosis of gastrointestinal acute GVHD is a clinical one, made on the basis of typical clinical findings. A biopsy of gastrointestinal mucosa is usually performed as a confirmatory procedure, and it may be particularly helpful for excluding alternative or coexisting pathologies such as neutropenic colitis, various infections, and drug toxicity (5,27,35). Acute GVHD of the gastrointestinal tract commonly manifests with a patchy distribution of lesions, and it can affect only a short segment of the digestive

tract (36–38). This type of distribution can lead to false-negative endoscopic and pathologic findings. Biopsies should therefore be obtained from multiple sites to increase the sensitivity of the procedure. Biopsy samples from the distal colon usually yield the highest sensitivities for detecting acute GVHD (27,37,38). Furthermore, the interpretation of gut biopsies can sometimes be challenging, as many overlapping histological features can occur as a result of chemotherapy or drug toxicity (39).

The most important finding in mucosal samples in patients with gastrointestinal aGVHD is the apoptosis of intestinal crypt epithelial cells (40,41). The apoptosis of cells in intestinal crypts is used as the histological hallmark of the mild disease. More-severe gastrointestinal aGVHD presents with cystically dilated crypts, and with crypt destruction and loss, up to compete mucosal denudation (41). Histological findings are typically graded, and the most frequently used histologic grading system is the one proposed by Lerner in 1974 (42) with certain modifications (41). Lerner's original grading system is shown in Table 2.

Table 2. Histologic grading of acute GVHD (Lerner grading system)

Grade	Histological findings:
I	Single-cell necrosis of epithelial cells
II	Necrosis and loss of glands
III	Focal microscopic mucosal denudation
IV	Diffuse mucosal denudation

Nowadays, gastrointestinal aGVHD is histologically usually categorized by the degree of crypt damage. Isolated apoptotic bodies without crypt loss are classified as Grade I, loss of individual crypts is found in Grade II, loss of multiple crypts in Grade III, and extensive crypt loss and epithelial denudation are typical findings of Grade-IV gastrointestinal aGVHD (40,41). The National Institutes of Health (NIH) Pathology Working Group recommends that all pathology reports for gastrointestinal GVHD should include both histologic features and a final diagnosis (43). According to the level of confidence provided by the histologic findings, histopathologic specimens should be reported in one of three categories: no GVHD, possible GVHD, and likely GVHD (43).

The clinical utility of histological grading systems remains unclear. Attempts to predict the prognosis or the response to treatment based on histological grade have been mostly unsuccessful (35). However, some more recent studies, such as one published in 2017 by Narkhede et al. (39), suggest that histological grading scores may play a role in predicting severity, treatment response, and outcome of acute gastrointestinal GVHD. Authors reported a positive correlation between the histological grade of GVHD, its clinical presentation, and non-relapse mortality (NRM) (39).

1.7. Early diagnosis of GVHD, prognostic scores and biomarkers

As patients with acute GVHD usually display clinical signs and symptoms at advanced stages in the development of mucosal injury, there is considerable interest in the early identification of individuals at high risk of developing severe forms of the disease. This has led to the development of a variety of clinical scores, while novel clinical and biochemical prognostic biomarkers are being constantly developed.

The classical grading of acute GVHD proposed by Glucksberg (13) has been shown to be predictive of the GVHD outcome. However, as it was developed using a limited number of patients before the modern era of allogeneic transplantation, various groups have tried to improve on it. One of the most notable additions to the GVHD scoring – the Center for International Blood and Marrow Transplant Research (CIBMTR) severity index (44) – groups patients with acute GVHD into four risk categories, based on the different patterns of organ involvement. The CIBMTR

severity index has shown better predictive value for patient survival, when compared with the classical Glucksberg grading, and was predictive of both response to treatment and treatment-related mortality (44).

A novel GVHD risk score – the Minnesota GVHD risk score – is based on the number of organs involved and the severity of GVHD at the onset of systemic steroid treatment. It has been developed and validated by MacMillan and her group (45–47). MacMillan was able to consistently identify patients with poorly responsive, high-risk acute GVHD based on the number of organs involved and the individual organ stage. These high-risk patients were three times less likely to respond to the steroid therapy and had a more than twofold increased risk of overall mortality and transplant-related mortality (46). Clinical scores such as the CIBMTR severity index and the Minnesota GVHD risk score proved to be able to identify high-risk populations when measured at disease onset.

In addition to the clinical scores, there is a growing field of novel biomarkers for early recognition of patients at high risk of developing severe and potentially lethal gastrointestinal GVHD. Biomarkers have been shown to be able to help recognize patients at risk of developing acute GVHD (48,49), or help diagnose the disease at its earlier stages (24). Biomarkers are used to identify patients at high risk of developing severe GVHD (50–53) and to predict the response to the therapy (51,52,54,55). Hartwell, for example, demonstrated in his recent study that the graft-versus-host reaction was already underway by day seven after the HSCT, and he could consistently identify a group of patients at high risk for developing lethal GVHD using a two-biomarker model (48). Recognizing GVHD before its onset could allow for more effective administration of primary or even preemptive therapy. Biomarker-directed interventions could be applied in the first days after the HSCT, much before clinical symptoms occur. It should be noted, however, that biomarkers for gastrointestinal GVHD are yet to be routinely used in everyday clinical practice, despite the progress achieved in recent years and the biomarkers' apparent potential to improve clinical outcomes in selected patients.

1.7.1. Regenerating islet-derived protein 3 alpha (Reg3alpha)

One of the most promising markers of gastrointestinal GVHD is regenerating islet-derived protein 3 alpha (Reg3alpha) (56). Known for its bactericide activity (57), Reg3alpha is produced by Paneth cells in the intestinal crypts. In addition to its antimicrobial activity, it plays a major role in reducing inflammation, in protecting intestinal stem cells, and in preventing gastrointestinal epithelial damage (18,24,50,58). As previously discussed, the immune-mediated destruction of intestinal stem cells and Paneth cells by activated donor T cells is the pathophysiological hallmark of gastrointestinal GVHD. A local inflammatory response and associated mucosal damage cause microscopic breaches in the epithelial barrier, permitting a transmission of Reg3alpha from the intestinal lumen into the systemic circulation (50). Ferrara showed that plasma concentrations of Reg3alpha were threefold higher in patients at the onset of acute gastrointestinal GVHD when compared with patients without GVHD and with non-GVHD enteritis (50). Furthermore, higher Reg3alpha concentrations at aGVHD onset predicted response to the treatment (50). These findings have been prospectively confirmed within the MAGIC International Consortium (48,52).As acute GVHD of the gastrointestinal tract commonly manifests with a patchy distribution of lesions, biomarkers could prove to be especially valuable in its assessment. The Reg3alpha levels (measured in the blood) can serve as a “liquid biopsy” that quantifies crypt damage and the loss of intestinal stem cells and Paneth cells (50,58). Such an estimate of the total damage to the mucosal barrier may also help to explain the prognostic value of Reg3alpha with respect to therapy responsiveness and mortality associated with GVHD (50).

1.7.2. EASIX score

Endothelial function and its vulnerability are important components of the pathogenesis of GVHD and its refractoriness to steroids (59–64). In their recent study, Luft and colleagues (59) developed an interesting clinical score – The Endothelial Activation and Stress Index (EASIX) – for easy assessment of endothelial damage in patients after allogeneic HSCT. EASIX is calculated with a simple formula using a combination of routinely monitored laboratory biomarkers: serum LDH value (expressed in U/l) multiplied by serum creatinine value (expressed in mg/dL) and divided by platelet count (expressed in 10^9 cells per liter). The authors showed that

there was a statistically significant correlation between the values of the EASIX score and overall survival and non-relapse mortality in patients after allogeneic HSCT, when measured at the time of diagnosis of acute GVHD. In another study by the same research group (60), the increased EASIX score values measured prior to the conditioning therapy significantly correlated with reduced overall survival and increased non-relapse mortality. These findings further confirm the importance of endothelial vulnerability in the development of acute GVHD. However, it must be noted that all three laboratory parameters used for calculation of the EASIX score (serum LDH, serum creatinine, platelet count) are only indirectly associated with endothelial damage. The low value of the hazard ratio in the study performed by Luft et al. suggests that, despite the demonstrated statistical significance, EASIX captures only part of the complex pathophysiology of acute GVHD.

The main advantage to the EASIX score is its easy applicability in daily practice. As part of this dissertation, the score was calculated in all patients using laboratory findings measured on the day of the gastrointestinal biopsy. It was later examined whether the EASIX score values (when measured before the onset of the clinical symptoms of acute GVHD) correlated with overall survival and non-relapse mortality.

1.7.3. The role of an early gut biopsy

Since 2005 a program of early-screening gastrointestinal biopsies has been implemented at the University Hospital in Regensburg. These early-screening biopsies were performed in patients showing no gastrointestinal symptoms at all, or – to clarify – mild gastrointestinal symptoms that did not meet the clinical criteria for the diagnosis of GVHD. Preliminary data indicated that increased apoptosis, one of the hallmarks of gastrointestinal GVHD, can be histopathologically demonstrated in up to a quarter of these tissue samples. As part of the work on this dissertation, the results of all gastrointestinal biopsies from the screening program were collected and then analyzed in terms of their prognostic significance (degree of later-developed GVHD – six-month, one-year and two-year non-relapse mortality). This is the first study to explore the use of an early biopsy in early diagnosis and risk stratification for gastrointestinal aGVHD.

2. Materials and methods

2.1. Selection process and inclusion criteria

All the patients in this retrospective study underwent allogeneic HSCT for malignant and non-malignant hematologic diseases at the University Hospital in Regensburg between 2005 and 2014. Data on all the performed biopsies of the upper and lower-gastrointestinal tract were collected. The biopsies performed within the first 100 days after the allogeneic HSCT were then further examined. The 100-day mark was chosen because of the research focus on acute gastrointestinal GVHD, which typically occurs within this time frame. Patients reported to have undergone a gut biopsy without presenting with symptoms indicative of, or characteristic for, gastrointestinal GVHD were included in the study cohort after careful examination of the transplantation and follow-up records. Patients showing no gastrointestinal symptoms at the time of the biopsy and patients who were documented as having received an early-screening biopsy were also included in the study cohort.

All the patients selected had been informed about the possible side effects of the gut biopsy and informed consent was obtained. Approval for this study was granted by the University of Regensburg Ethics Committee Review Board.[1]

2.2. Exclusion criteria

Patients with an already-existing clinical or histological diagnosis of gastrointestinal GVHD were excluded from the study. Documented active GVHD of skin or liver was also an exclusion condition. Patients who had recently started treatment with corticosteroids or any other immunosuppressive therapy were excluded from the cohort. The decision to start the immunosuppression was interpreted as indicative of clinical suspicion of GVHD, and therefore these patients were not included in the study. Escalation of the dos of immunosuppression shortly before or after the biopsy was not allowed. However, the continued low-dose prednisolone treatment, which was being tapered off at the time of the biopsy, was not prohibited.

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[1] Institutional approval no. 09/059: *Untersuchung zur intestinalen Immundysregulation nach allogener KMT an Biopsien (Darmbiopsien).*

2.3. Study cohort and subgroups

Based on the previously discussed inclusion and exclusion criteria, a total of 108 patients were selected for the study. They were divided into two subgroups. The first subgroup consisted of the 26 patients who received a gut biopsy in the complete absence of any gastrointestinal symptoms. A total of 27 biopsies were performed in this subgroup (one patient received both an upper- and lower-gut endoscopy).

The second, larger subgroup, consisted of 82 patients who presented with mild gastrointestinal symptoms not sufficient for the clinical GVHD diagnosis. In total, 90 biopsies were performed on these patients (eight patients received both an upper- and lower-gut endoscopy). All statistical calculations were performed on the full patient collective (108 cases), on the no-symptoms subgroup (26 cases), and on the symptomatic subgroup (82 cases).

If the patients received both an upper- and lower-gastrointestinal biopsy (usually performed on the same day) the histological results of these biopsies were combined. Two negative biopsies were documented as a negative finding. A single- or double-positive biopsy was documented as a positive finding.

2.4. Patient collective

The study cohort consists of 108 patients, 68 of whom are males (63.0%) and 40 females (37.0%). The average age at HSCT was 51.5 years (SD 10.9). The youngest patient was 22 at the time of allogeneic HSCT and the oldest transplanted patient was 70. Age and gender distributions across the subgroups are shown in Table 3.

Table 3. Age and Gender Distribution Across the Study Cohort

	No GI symptoms		Mild GI symptoms	
Gender	Number	Percent	Number	Percent
Male	17	65.4	51	62.2
Female	9	34.6	31	37.8

Age (in years)	53.3 (SD 9.8)		50.9 (SD 11.1)	
18–49	7	26.9	30	36.6
50–70	19	73.1	52	63.4

The most frequent diagnosis in the patient population was acute leukemia, found in 66 patients (61.1%). This was followed by non-Hodgkin lymphoma, which was found in 24 patients (22.2%). Seven patients had myelodysplastic syndrome (6.5%) and six had myeloproliferative syndrome (5.6%). Three patients (2.8%) had Hodgkin lymphoma and aplastic anemia was found in two (1.9%). The distribution of the primary disease was similar among the two subgroups, as shown in the Table 4.

Table 4. Distribution of Patients Between the Two Subgroups (No Gastrointestinal Symptoms and Mild Gastrointestinal Symptoms) According to the Primary Disease

	No GI symptoms		Mild GI symptoms	
Diagnosis	Number	Percent	Number	Percent
Acute Leukemia	13	50.0	53	63.9
Non-Hodgkin Lymphoma	9	34.6	15	18.1
Myelodysplastic Syndrome	2	7.7	5	6.0
Myeloproliferative Syndrome	1	3.8	6	7.2
Hodgkin Lymphoma	0	0	3	3.6
Aplastic Anemia	1	3.8	1	1.2

Most of the patients (48 patients – 44.4%) were in an advanced stage of their disease at the time of allogeneic HSCT. Thirty patients (27.8%) were in the intermediate stage of the disease. The same number of patients (30 patients – 27.8%) received allogeneic HSCT in the early stage of their disease. The two subgroups (no gastrointestinal symptoms and mild gastrointestinal symptoms) showed a similar distribution with regard to the pretransplantation disease severity, as shown in Table 5.

Disease status at the time of transplantation was classified as low, intermediate, and high, as defined by the American Society of Blood and Marrow Transplant (ASBMT) guidelines.

Table 5. Distribution of Patients Between the Two Subgroups According to the Stage of the Disease

	No GI symptoms		Mild GI symptoms	
	Number	Percent	Number	Percent
Early stage	6	23.1	24	29.3
Intermediate stage	8	30.8	22	26.8
Advanced stage	12	46.1	36	43.9

In total, 100 patients (92.6%) had received peripheral blood stem cells as a stem-cell source. Seven patients (6.5%) had received bone marrow, and one patient (0.9%) had received cord blood as the stem-cell source. There were no significant differences between the two subgroups.

The majority of donors (83 donors, 76.8%) were unrelated, while 25 donors were related to patients (23.2%), two of them being only haploidentical. Patients received GVHD prophylaxis regimens depending on the institutional standard at the time of the transplantation or the requirements of the transplantation protocol. Prophylaxis generally consisted of a calcineurin inhibitor in combination with methotrexate or mycophenolate. In the cases of unrelated donors, ATG was added.

Most of the patients (89 patients, 82.4 %) received a reduced-intensity preparative regimen (RIC). Myeloablative conditioning was used in 19 cases (17.6%). There was no difference between the subgroups regarding the preparative regimen (*P* =.734).

The majority of patients (80 out of the 108) included in this study did not receive systemic corticosteroids. In the subgroup showing no gastrointestinal symptoms, only three patients received low-dose prednisolone, while 25 out of 82 patients showing mild gastrointestinal symptoms received systemic corticosteroids, which were being tapered off at the time of the biopsy. The most common reason for the introduction of steroids was engraftment syndrome after the allogeneic HSCT, while some patients had had previous episodes of cutaneous GVHD.

2.5. Data collection

2.5.1. Demographic and transplantation-related data

Data was collected from already-existing transplantation and follow-up documentation. These records contain all relevant demographic data and comprehensive HSCT-related information including the condensed clinical assessment of the patient with a commentary on the possible GVHD signs and symptoms. Clinical evaluations were performed daily for the stationary patients and at every follow-up visit for the outpatients (usually twice a week in the first months after the transplantation). Residents completed the evaluations under direct supervision from attending physicians in the inpatient and outpatient units.

2.5.2. Histology

Histological assessment of specimens was performed at the Institute of Pathology at the University Hospital in Regensburg. Because of the retrospective nature of this study, biopsies were reported by several pathologists. GVHD assessment and grading were performed by Dr. Elisabeth Huber and Dr. Katrin Hippe on the basis of the criteria of the National Institute of Health consensus, using a modified system of a grading scheme published by Lerner in 1974 (42).

2.5.3. Reg3alpha

For this research, already-existing data on serum values of Reg3alpha was used. The samples were collected as part of an ongoing prospective Mount Sinai Acute GVHD International Consortium (MAGIC) study, approved by the institutional ethics committee.[2] In 72 patients, Reg3alpha serum levels were analyzed in duplicate at the Holler Lab using indirect ELISA within the seven days before or after the day of the gastrointestinal biopsy. The mean was calculated from the duplicates. The values are expressed in ng/mL. Data was not available for 36 patients.

2.5.4. EASIX

Laboratory values needed for calculating the EASIX score (LDH, creatinine and platelet count) were collected from the transplantation and follow-up documentation and were available for all 108 patients included in the study. Laboratory check-ups are routinely performed at every patient visit and the results are well documented. Values of LDH (expressed in U/l), creatinine (expressed in mg/dL) and the platelet count (expressed in 10^9 cells per liter), measured on the day of the gastrointestinal biopsy, were collected. For patients who needed a platelet transfusion before the biopsy, the initial value, measured before the transfusion, was used. The value of the EASIX score was calculated using the prediction tool (the calculator) developed by Luft and colleagues (59). This calculator is freely available on the website of the German Cancer Research Center (DKFZ) via this link http://biostatistics.dkfz.de/EASIX/.

[2] Institutional approval no. 14-101-0047, Amendment 14-47_1-101: Begleituntersuchungen im Rahmen der deutschen Zentren des MAGIC Konsortiums (Mikrobiomuntersuchungen incl 16s rRNA in Stuhl und Urin)

2.6. Statistics

The statistical evaluation was performed with version 22 of the IBM SPSS program. Comparisons of categorical data were analyzed using Pearson's chi-square test or with Fisher's exact test, when appropriate. Survival analysis was performed using the Kaplan–Meier method. A log-rank test was used to statistically compare the curves and the p-value was shown. To estimate the impact of the independent variables on treatment-related mortality, a Cox regression analysis was used. The patients who were still alive at the last follow-up have been censored. Risk factors for non-relapse mortality were analyzed using univariable and multivariable proportional cause-specific hazards regression.

For the primary statistical analysis of EASIX, the log2 transformed index, log2(EASIX) = log2(LDH) + log2(creatinine) – log2(thrombocytes) was used. Cause-specific hazard ratios were computed to describe the prognostic effect of EASIX. LDH, creatinine, and thrombocyte counts were tested for the combined patient cohort (N = 108) in a multivariate Cox regression model with endpoint NRM in order to assess the prognostic value of the single EASIX components.

Statistical significance was considered for $P < .05$. All P values reported are two-sided.

3. Results

3.1. Histological findings

A total of 117 biopsies were performed in 108 patients. Nine patients received both upper- and lower-gut endoscopy. Overall, 46 upper-gut biopsies were performed, and 71 lower-gut biopsies. The median number of days between HSCT and the time the gut biopsy was performed was 24.8 (range 11–83, SD 12.5). There was no statistically significant difference between the two subgroups (no symptoms vs. mild symptoms) as regards this parameter (*P* =.15).

A total of 27 patients had histological findings consistent with gastrointestinal GVHD. Findings were graded according to the Lerner classification – Grade I was found in 25 cases, and Grade II in two cases. No higher-grade gastrointestinal GVHD was found in these screening biopsies. In the subgroup of asymptomatic patients four (16.4%) had histological findings consistent with GVHD (three with Lerner Grade I, one with Grade II). In the subgroup of patients with mild gastrointestinal symptoms, 23 out of 82 (28.0%) had histological signs of gastrointestinal GVHD. Lerner Grade I was found in 22 cases, and Lerner Grade II in one case. Although positive histological findings were more common in the group of patients with mild gastrointestinal symptoms when compared with the patients with no symptoms (28.0% vs. 16.4%), this difference was not statistically significant (*P* =.13). The prevalence of positive histological findings in early gastrointestinal biopsies is depicted in Graph 2.

The next step examined whether any of the demographic or transplantation-related parameters influenced the prevalence of positive histological findings in the screening biopsy of the gut. No statistically significant correlations were found, as summarized in Table 6.

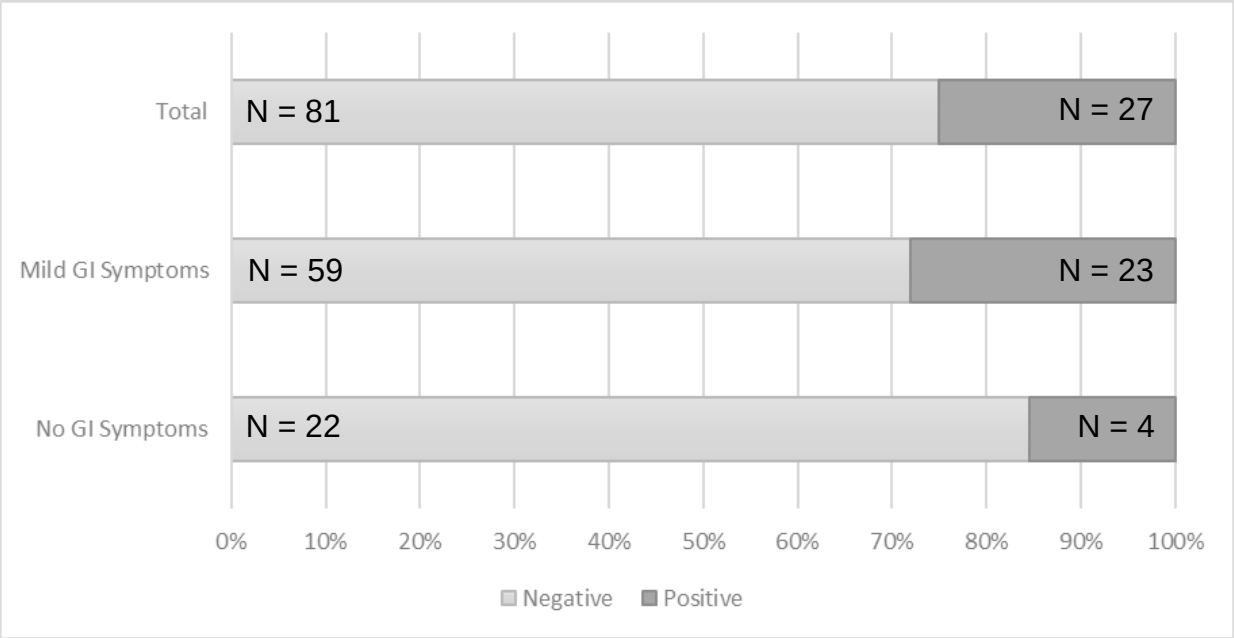
Table 6. Influence of Demographic and Transplantation-Related Risk Factors on the Prevalence of Histological GVHD in the Early Biopsy of the Gut Mucosa

		Biopsy findings		
Risk factors		No GVHD	GVHD	*P*
Gender	Male	17	51	
	Female	28	12	.571
Age	50 years and older	50	20	
	Younger than 50	29	9	.584
Stage of the disease	Early	25	5	
	Intermediate	18	12	
	Late	36	12	.116
Comorbidity index	0–2	39	16	
	> 2	25	8	.621

Karnofsky index	90% or more	63	19	
	80% or less	16	9	.201
Preparative regimen	Reduced intensity	65	24	
	Myeloablative	14	5	.954
Donor relationship	Related (+ Haplo)	19	6	
	Unrelated	60	23	.761
Donor – Gender	Male	53	20	
	Female	25	8	.776
Graft source	PBSCT	72	28	
	BMT	6	1	
	Cord blood	1	0	.607

Abbreviations: PBSCT, peripheral blood stem-cell transplantation; BMT, bone-marrow transplantation

Graph 2. Prevalence of Positive Histological Findings in Early Gastrointestinal Biopsies (Absolute Number of Cases and Percentages)

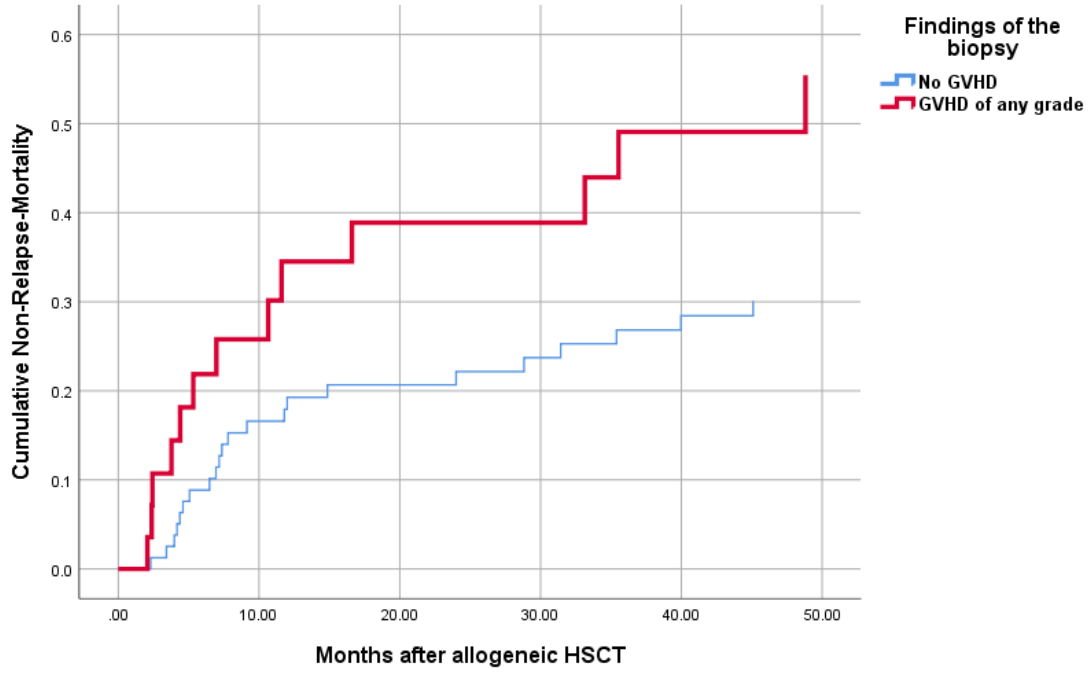


3.1.1. Histological findings and non-relapse mortality (NRM)

Data on non-relapse mortality (NRM) was collected from the already-existing database that was last updated on January 31, 2018. Positive findings of the early-screening biopsy (consistent with gastrointestinal GVHD) correlated with NRM (*P* =.024) as depicted in Graph 3.

Positive findings in the gastrointestinal biopsy correlated with increased NRM at six months (25.0% versus 8.9%; *P* =.03) and 12 months (35.7% versus 17.7%, *P* =.04). NRM at 24 months was more common in patients with positive biopsy findings (35.7% versus 20.3%; *P* =.10), albeit without statistical significance.

Graph 3. The Dependence of Cumulative NRM on the Results of the Early-Screening Biopsy



When analyzed in all 108 patients, using the Cox proportional-hazards model, it was demonstrated that histological findings consistent with GVHD predict six-month NRM (Hazard ratio [HR] = 4.15; 95% confidence interval [CI] = 1.38–12.53) and 12-month NRM (HR = 2.96; 95% CI = 1.27–6.87). The results of the Cox proportional-hazards model for NRM at six and 12 months are shown in tables 7 and 8. Besides the results of the screening biopsy, the stage of the disease showed to be predictive of non-relapse mortality at six months. None of the other parameters (initial diagnosis, gender, age at HSCT, Karnofsky index, and the prolonged use of antibiotics) showed to be predictive of the NRM at six and 12 months after the HSCT.

When analyzed individually, in both subgroups (patients with no gastrointestinal symptoms and patients with mild gastrointestinal symptoms), histologically diagnosed GVHD remained a statistically significant predictor of six-month NRM and 12-month NRM.

Presenting with gastrointestinal symptoms itself was not a predictor of NRM as there was no statistically significant difference in mortality in relation to this parameter.

Table 7. Results of Cox Regression Model for NRM at Six Months in Patients Undergoing a Screening Gastrointestinal Biopsy Post–HSCT

Variable	HR	95% CI	*P*
Results of the screening biopsy (with GVHD vs. without GVHD)	4.15	1.38–12.53	.011
Diagnosis (Acute Leukemia vs. others)	0.97	0.30–3.07	.965
Stage of the disease (late vs. early)	3.74	1.12–12.67	.032
Gender (male vs. female)	1.54	0.48–4.85	.460
Age (younger than 50 vs. older than 50)	1.53	0.40–5.8	.533
Antibiotics (yes vs. no)	1.77	0.38–8.07	.460
Karnofsky (≥ 90% vs. ≤ 80%)	1.09	0.28–4.23	.892

Table 8. Results of Cox Regression Model for NRM at 12 Months in Patients Undergoing a Screening Gastrointestinal Biopsy Post–HSCT

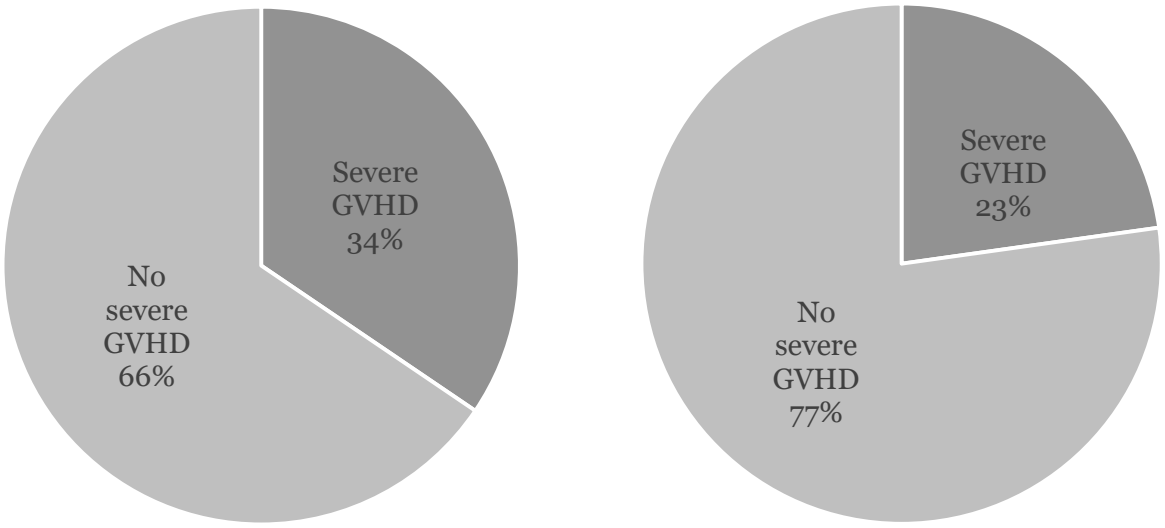
Variable	HR	95% CI	*P*
Results of the screening biopsy (with GVHD vs. without GVHD)	2.96	1.27–6.87	.012
Diagnosis (Acute Leukemia vs. others)	0.88	0.36–2.13	.782
Stage of the disease (late vs. early)	2.33	0.96–5.68	.062
Gender (male vs. female)	1.40	0.59–3.36	.441
Age (younger than 50 vs. older than 50)	1.00	0.38–2.61	.997
Antibiotics (yes vs. no)	0.96	0.35–2.67	.95
Karnofsky (≥ 90% vs. ≤ 80%)	0.69	0.26–1.80	.567

3.1.2. Maximal degree of later-developed GVHD

The next step examined whether the maximal degree of the later-developed GVHD correlated with the findings of the screening gastrointestinal biopsies. More than one-third (34.48%) of patients with positive findings in the screening biopsy (histologically consistent with GVHD) developed severe GVHD (clinical Grade III or Grade IV) at one point during their follow-up. In comparison, severe GVHD developed in 22.78% of patients with negative findings in the screening biopsy. There was no statistically significant difference between the two groups (chi square = 1.512, *P* =.219). Similarly, development of severe *gastrointestinal* GVHD was not more common in patients with positive results in the screening biopsy of the gut (chi square = 0.732, *P* =.392). The results are depicted in Graph 4.

Graph 4. Maximal Degree of Later-Developed GVHD Depending on the Results of the Screening Biopsy

A: Positive Biopsy **B: Negative Biopsy**



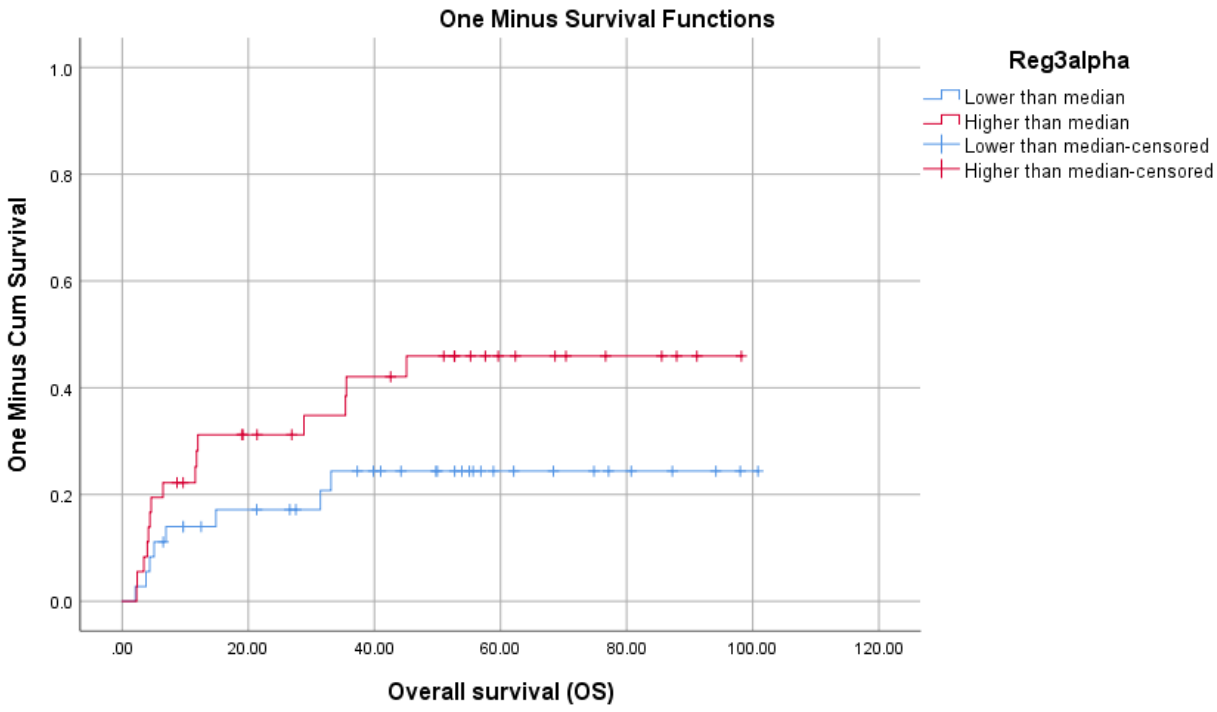
Reg3alpha values were measured in 72 patients, as described in Chapter 2.5.3. The patient collective is described in Chapter 2.4. The mean value of Reg3alpha was 94.5 ng/mL (minimum 4.4, maximum 2296.0, SD 279.7). The median concentration of Reg3alpha was 32.4 ng/mL.

The presence of mild gastrointestinal symptoms at the time of the screening biopsy was associated with higher Reg3alpha levels (114.8 ng/mL vs. 45.2 ng/mL); however, the increase in levels were not statistically significant (Z= −0.973; *P* =.331).

Plasma Reg3alpha concentrations at the time of the screening biopsy could not distinguish between the patients whose gastrointestinal biopsies showed evidence of GVHD (increased apoptosis, consistent with histologic Grade I) and patients with negative biopsy findings.

In the next step, for analysis of the Reg3alpha impact on overall survival and mortality, the patient cohort was divided into two equal groups based on the median Reg3alpha concentration: high (> 32**.**4 ng/mL) and low (≤ 32.4 ng/mL). Distribution of non-relapse mortality in these two subgroups is depicted in Graph 5. Although a tendency toward increased NRM was noticed in the subgroup with higher Reg3alpha values, this difference was not statistically significant (log rank chi square 2.918, *P* =.088).

Graph 5. Dependency of Cumulative NRM on the Reg3alpha Value (Higher Than Median Vs. Lower Than Median), Measured at the Time of Screening Biopsy



The association of elevated (higher than median) biomarker levels at the time of the screening biopsy with increased non-relapse mortality did not reach a statistically significant level. However, a tendency was observed (*P* =.088), as NRM was more commonly found in patients with higher than median Reg3alpha levels.

Similarly, there was no correlation between Reg3alpha concentrations at the time of the screening biopsy and the eventual maximal clinical stage of gastrointestinal GVHD. The tendency toward increased non-relapse mortality and the later development of severe GVHD was, however, observed in patients with the highest Reg3alpha levels. GVHD clinical Grade III or IV was more common in patients with Reg3alpha values in the upper quartile, as eventually 16.7% of them developed severe GVHD, compared with only 5.5% in other patients. This difference did not reach a statistically significant level. Similarly, patients with Reg3alpha values in the upper quartile had a 33% chance of death due to transplantation-related complications in the first two years after the HSCT, compared with 18% in the rest of the cohort. There was an observed difference between the two subgroups, but it was not statistically significant (*P* =.19).

3.3. Predictive value of the EASIX score

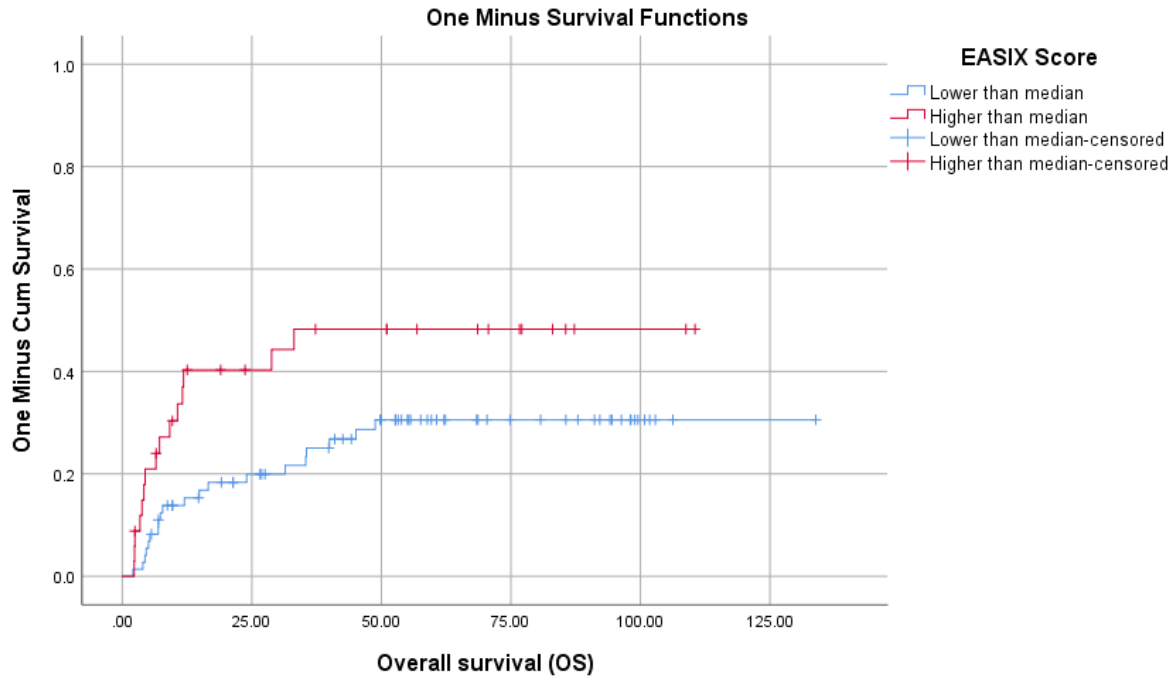
The EASIX score was calculated in all patients, using laboratory values measured on the day of the screening biopsy, as already described in Chapter 2.5.4. The patient collective is described in Chapter 2.4. The mean value of the EASIX score was 9.80 (minimum 0.74, maximum 72.50, SD 12.39).

The presence of gastrointestinal symptoms did not correlate with the value of EASIX as there was no difference between the patients with no symptoms and the patients with mild symptoms at the time of the biopsy (*P* =.15). The EASIX score was higher in patients with positive histological findings in their screening biopsy (consistent with gastrointestinal GVHD), although this difference was not statistically significant (11.10 vs. 8.60, *P* =.30).

Non-relapse mortality (NRM) was increased in patients with a higher than median EASIX score (log rank chi square = 4.91, *P* =.027). These findings are depicted in Graph 6. The predictive value of the EASIX score for six-month NRM was not found,

despite the tendency observed (HR = 2.47; 95% CI = 0.84–7.28; *P* =.099). EASIX was, however, predictive for 12-month NRM (HR = 3.09; 95% CI= 1.34–7.11; *P* =.008), and for the 24-month NRM (HR = 2.64; 95% CI= 1.22–5.70; *P* =.013).

Graph 6. Cumulative Non-Relapse Mortality in Relation to EASIX Score (Higher Than Median Vs. Lower Than Median), Measured at the Time of the Screening Biopsy



The next step investigated whether the EASIX score is associated with the later development of severe GVHD (Grade III and Grade IV). Although severe GVHD was more common in patients with higher EASIX values, this difference was not found to be statistically significant.

To assess the prognostic value of the single EASIX components, LDH, creatinine and thrombocyte counts were tested in a multivariate Cox regression model with non-relapse mortality as the endpoint. Taken separately, none of these three components was significantly associated with the hazard of non-relapse mortality, although a tendency was visible regarding the value of thrombocytes (*P* =.07).

4. Discussion

4.1. Histology

4.1.1. Incidence of positive GVHD findings

In this retrospective analysis, a quarter of allogeneic HSCT recipients presenting with none or with mild gastrointestinal symptoms had histological findings consistent with acute gastrointestinal GVHD in the early-screening gut biopsy – performed in the first weeks following the allogeneic HSCT. In almost all cases, no histological signs of advanced GVHD were found, and the vast majority of the findings were classified as Grade I according to the Lerner classification – the apoptosis of single epithelial cells being its hallmark feature. Clinicians must be aware that low-grade histological changes in the gut epithelium can be caused by a multitude of conditions in the post–HSCT setting – e.g., the effects of chemotherapy or infections – before initiating therapy with high-dose steroids. The screening gut biopsy in this study was performed after a median of 25 days after the allogeneic HSCT. As changes in the gut epithelium caused by the conditioning chemotherapy usually happen in the first two weeks after the treatment, false-positive findings due to chemotherapy toxicity were unlikely. Similarly, routinely performed microbiological evaluations of stool samples (including CMV) limited the number of false-positive histological findings caused by gastrointestinal infection.

A total of 26 patients who showed no gastrointestinal symptoms at the time of the screening biopsy were included in this study. Interestingly, early mucosal changes, consistent with acute gastrointestinal GVHD, were found in four cases, representing 16% of this subgroup. High-dose corticosteroid treatment was not initiated with these four patients because of the lack of any gastrointestinal symptoms. Unfortunately, all four patients developed symptomatic GVHD in the later follow-up and died because of its complications.

Although the incidence of the histological (low-grade) acute gastrointestinal GVHD in asymptomatic or mildly symptomatic patients was found to be relatively high in this study, it is reasonable to assume that its occurrence has not been overestimated. Systemic inflammation in a post-allogeneic-HSCT setting is a ubiquitous finding and it has been suggested that virtually all allograft recipients experience graft-vs.-host reactions, but that not all develop clinically significant disease (51). Furthermore, the

early biopsies, when done immediately after the onset of the symptoms and signs of presumptive GVHD, may produce a false negative because of subtle and focal morphological changes in the gastrointestinal mucosa (43). Low-grade microscopical changes that occur on the healthy-looking gastrointestinal mucosa have already been commonly described. In the single-center study, Thompson and colleagues reported that histological changes consistent with acute GVHD were found in 44.7% of endoscopically normal biopsy sites (37). Similar findings were reported by Ross and colleagues, as more than 60% of healthy-looking mucosal biopsy sites show microscopical changes consistent with acute gastrointestinal GVHD (38).
Although the histological assessment alone cannot be considered to be the gold standard for diagnosis of gastrointestinal GVHD because of sampling error and the patchiness of GVHD-related abnormalities (26), an early gastrointestinal biopsy should be included in the diagnostical algorithm in low-symptomatic patients, and it should be considered even in patients reporting no gastrointestinal symptoms.

4.1.2. Predictive value of the screening biopsy

This study shows that positive results of the early-screening biopsy of the gut mucosa (histological findings consistent with acute gastrointestinal GVHD) correlated with an increased non-relapse mortality at six months (25.0% vs. 8.9%; HR = 4.15) and 12 months (35.7% vs. 17.7%; HR = 2.96) when compared with the patients with negative biopsy results, regardless of the presence of gastrointestinal symptoms.

The prognostic value of the histological findings and grading scores in the post-allogeneic-HSCT setting, and their correlation with the clinical outcomes, hasn’t been thoroughly investigated. The existing data is limited and inconsistent, and it focuses predominantly on patients with advanced histological changes, while the impact of early histological GVHD remains poorly understood. The presence of advanced changes in gastrointestinal mucosa has been demonstrated to correlate with increased transplant-related mortality in several retrospective single-center studies (39,61,62). For instance, Melson and colleagues reported that advanced histological GVHD of the gastrointestinal tract (Grade III or IV according to the Lerner classification) was associated with a higher clinical stage and with steroid-refractory disease (61). Similar results were reported in a more recent study (39) that demonstrated that a higher histologic grade correlated well with the higher clinical

grade and was prognostic of non-relapse mortality as well as significantly associated with the treatment response. No correlation between the histological grade and the clinical severity was found, however, in the study published by Abraham and colleagues. (62). Advanced histological changes – Grade IV according to the Lerner classification – were reconfirmed to independently predict a poor outcome (62).

4.1.3. The importance of early histological changes

In total, 25 out of 27 patients (92.6%) with positive findings in the gastrointestinal-screening biopsy were diagnosed with the early histological changes – Grade I according to the Lerner classification. These results should not be surprising, especially for the screening biopsies, as GVHD nowadays is typically diagnosed in its earlier stages. Advanced changes in the gut mucosa (Lerner Grade II, III, or IV) are considered to be a failure to adequately control GVHD, and they may require extraordinary therapeutic measures (35).

It is not uncommon in everyday clinical practice for low-grade histological changes to not lead to the initiation of the treatment, especially in patients who are mildly symptomatic, as GVHD continues to be a diagnosis based predominantly on the presence of clinical symptoms. This study shows, however, that both asymptomatic and mildly symptomatic patients who do show early histological changes have increased mortality when compared to their counterparts with negative biopsy results. These results resonate well with a recent single-center study published in 2017 by Im and colleagues (63). The authors reported that the gastrointestinal GVHD Grade I histology provided important prognostic information independent of the clinical stage and was associated with higher non-relapse mortality. Furthermore, histologic Grade I did not lessen the markedly adverse outcome of advanced lower-gastrointestinal GVHD in clinical stages III to IV. The authors recommend, like the findings of this study, that identification of Grade I histology should not be dismissed (and warrants treatment) as its presence is associated with increased non-relapse mortality.

4.1.4. Improving on Lerner?

Despite the growing amount of evidence that lower-grade histological changes in gut mucosa correlate well with increased non-relapse mortality, as demonstrated in this study, multiple questions remain open. There is no clear consensus on the exact threshold of minimal histological change that is sufficient for the diagnosis of gastrointestinal GVHD, as the interpretation of findings varies among pathologists from different transplant centers. Furthermore, the degree of injury described as histological Grade 1 according to the Lerner classification includes a broad spectrum of apoptotic activity from rare to numerous, falling just short of exploding crypts (43). This therefore creates difficulties in identifying the subset of patients with increased risk of developing life-threatening GVHD. Although histological GVHD in this study correlated with increased non-relapse mortality, a correlation with the later development of severe clinical GVHD was not found. The subset of patients remained low symptomatic or asymptomatic in further follow-up, and others developed high-grade GVHD. This difficulty in accurately predicting a low-prevalence condition such as severe gastrointestinal GVHD remains a common hurdle for all published early GVHD indicators and predictors.

The unpredictable correlation between low-grade histological changes, the later development of severe clinical GVHD, and non-relapse mortality may be attributed to the deficiencies of the Lerner grading system itself, as suggested in an interesting study published by Myerson et al. in 2017 (35). As the authors note, the Lerner classification system was developed in the early days of allogeneic HSCT, and it was mostly based on the results of autopsy findings in patients with severe gastrointestinal GVHD. This increased prevalence of advanced mucosal changes led to a certain one-sidedness to the system, which is weighted toward the more-advanced disease, a form that is not as common in the modern era of transplantation medicine. This apparent limitation of the Lerner classification in identifying gastrointestinal GVHD in its earliest stages led the authors to develop a new grading system that stratifies the low-level histological Grade I category into four activity grade categories, based on the average frequency of apoptotic cells. Good initial results of the new system seem to confirm the hypothesis that there is useful

information hidden in the Lerner Grade I category that could potentially guide immediately actionable treatment decisions (35).

Further methods for improving the predictive value of histological findings have also been suggested. Levine and colleagues reported in 2013 that the quantification of Paneth-cell numbers in the duodenum, which is easily accomplished with light microscopy, can aid in establishing the diagnosis of gastrointestinal GVHD and has prognostic importance (24). Lower numbers of Paneth cells at diagnosis correlated in their study with clinically more-severe GVHD and a lower likelihood of response to the immunosuppressive treatment (24). These findings were later confirmed in a more recent study by Weber and colleagues who reported a decrease in the Paneth-cell count in patients with severe gastrointestinal GVHD (64). However, it must be noted that the loss of Paneth cells seems to be a late occurrence in the pathophysiological cascade of gastrointestinal GVHD, and hence it may not be of paramount importance in its early recognition and diagnosis.

4.1.5. Histology – conclusions

To conclude this chapter, early histological changes in the gut mucosa provide valuable information in the post-allogeneic-HSCT setting, and histological assessment should be an integral part of a comprehensive diagnostic approach to patients presenting with any gastrointestinal symptoms. This study supports the idea of performing early-screening biopsies of gastrointestinal mucosa, especially in patients at high risk of developing GVHD, as the data implies that early histological changes predate the development of gastrointestinal symptoms in a significant subset of patients. An early preemptive approach and treatment initiation may therefore prevent later damage associated with advanced GVHD. Further prospective clinical trials are needed, and the optimal timing of an early-screening biopsy remains to be debated. Furthermore, the detection of patients at risk of developing severe GVHD based on the results of a single biopsy may prove to be challenging, as histopathology represents a one-time assessment of a complex and dynamic pathophysiology of mucosal injury.

4.2. Prognostic impact of Reg3alpha

In this retrospective analysis, plasma levels of regenerating islet-derived protein 3 alpha (Reg3alpha) that were collected at the time of the screening biopsy (no clinical signs of GVHD present) did not correlate with increased non-relapse mortality or with later development of severe gastrointestinal GVHD. These results stand in contrast to the growing amount of evidence that this biomarker has a predictive role both at the moment of GVHD diagnosis and prior to its onset, as early as in day seven after the post-allogeneic HSCT.

The initial, pivotal study that demonstrated the predictive potential of Reg3alpha at the time of GVHD onset was published by Ferrara and his research group in 2011 (50). Reg3alpha levels in plasma were shown in this study to be threefold higher in patients at the onset of gastrointestinal GVHD than in all other patients. Higher values of this biomarker were especially predictive of lower-gastrointestinal GVHD – this is arguably the most impactful complication of the allogeneic HSCT. Elevated Reg3alpha concentrations correlated with an increased one-year non-relapse mortality and inferior one-year overall survival; they could also successfully predict the response to the steroid treatment (50). Furthermore, Reg3alpha plasma concentrations were significantly higher in patients with advanced histological changes in the gut mucosa, and they correlated with the later development of severe clinical gastrointestinal GVHD. All these findings remained statistically significant after adjusting for known risk factors of donor type, degree of HLA match, conditioning intensity, age, and baseline disease severity (50). The results of multiple follow-up studies that reported a similar predictive strength of Reg3alpha will be discussed later in this chapter.

The discrepancy between the findings of Ferrara and his research group and the findings of this study could be explained to some extent in terms of the timing of the measurement of biomarker levels. In contrast to the Reg3alpha analysis, at the moment of the GVHD diagnosis – as made by Ferrara and colleagues – patients included in this cohort had their plasma Reg3alpha levels measured as part of the screening diagnostics, while not displaying signs or symptoms of gastrointestinal GVHD. The majority of the included patients never developed acute GVHD, and it can be argued that, at the moment of the measurement, they displayed significantly

less inflammation of gastrointestinal mucosa with consecutive destruction of Paneth cells. In turn, this would have influenced the plasma levels of Reg3alpha and the levels' prognostic strength. As already described, Reg3alpha is released into the bloodstream as GVHD damages the integrity of the intestinal mucosa and leads to the destruction of the Paneth cells in gastrointestinal crypts. Comparing the median values of plasma Reg3alpha in this study with the study by Ferrara and colleagues seems to confirm this hypothesis, as the values measured by Ferrara and his team are almost fivefold higher (151 ng/mL vs. 32 ng/mL).

Although not statistically significant, the tendency toward increased non-relapse mortality and later development of more-severe GVHD was observed in patients with increased Reg3alpha levels in the patient cohort from Regensburg too. For example, the patients with the values of Reg3alpha in the upper quartile had 33% chance of dying due to transplantation-related complications in the first two years after the HSCT, compared to an 18% chance in the rest of the cohort. Similarly, severe GVHD was more common in this subgroup of patients (22% compared with 7%). Presenting with mild gastrointestinal symptoms at the time of the screening biopsy also led to an increase in the average Reg3alpha levels (114.8 ng/mL vs. 45.2 ng/mL); however, it was not statistically significant (P =.168).

As previously mentioned, the predictive value of Reg3alpha at GVHD onset was validated by multiple research groups (52,55,64). Levine et at. (52) developed a prognostic score based on three biomarkers (Reg3alpha, TNFR1, and ST2) that was demonstrated to predict the cumulative incidence of non-relapse mortality when measured at GVHD onset. This biomarker-based score could also predict response to the treatment and, interestingly, the later development of gastrointestinal GVHD in patients who presented without gastrointestinal symptoms and who were initially diagnosed with GVHD of the skin (52). Similarly, Weber et al. reported that severe gastrointestinal GVHD correlated with higher serum concentrations of Reg3alpha (64). The biomarker-guided strategy was further examined for its predictive value in a large study by Major-Monfried et al. that included 507 patients (55). The authors demonstrated that a biomarker score based on the values of Reg3alpha and ST2, measured in the blood one week after the initiation of the systemic treatment of

GVHD, predicted long-term outcomes in steroid-resistant GVHD significantly better than the clinical criteria did.

The hypothesis that alloimmune inflammation of gastrointestinal mucosa predates the clinical symptoms of GVHD was tested by Hartwell et al. in the largest multicenter study on Reg3alpha to date, which included 1287 patients (48). The authors show that a two-biomarker algorithm (using concentrations of ST2 and REG3a), measured one week after HSCT and before the onset of GVHD symptoms, could successfully identify patients with increased cumulative incidence of six-month non-relapse mortality (28% in the high-risk group compared with 7% in the low-risk group; *P* <.001). These remarkable findings imply that a graft-versus-host reaction is already in progress by day seven and has led to increased biomarker concentrations, even though clinical symptoms may not occur until days or weeks later (48).

Despite the promising results of the above studies and the continuous development of new diagnostic and therapeutic approaches to acute GVHD, multiple challenges remain. Accurately predicting a low-prevalence condition such as severe gastrointestinal GVHD remains a common obstacle for all published early GVHD indicators. Despite being able to identify high-risk groups of patients, indicators of acute GVHD are not as efficient in ascertaining the risk for an individual patient. McDonald et al. suggest that, with the current sensitivity, specificity, and positive predictive value of the best indicators available, the number of false positives still outnumbers the number of true positives (51). This could lead to overtreatment of some patients who were not destined to develop more-severe GVHD, putting them at risk of additional complications associated with profound immunosuppression. False-negative biomarker-based predictions could, on the other hand, leave some of the high-risk patients unidentified and at risk of excessive morbidity and mortality rates.

Some research groups have disputed the predictive value of plasma levels of Reg3alpha. In a study investigating the impact of six different biomarkers measured before the initiation of GVHD treatment, Reg3alpha underperformed and did not corelate with increased non-relapse mortality or later development of high-grade GVHD (51). This research group identified plasma ST2 (a suppressor of tumorigenicity 2) and plasma TIM3 (T-cell immunoglobulin and mucin-domain

containing-3) as the strongest predictors of transplantation-associated complications. Similarly, Balakrishnan et al. show that values of ST2 (which is a negative regulator of type 2 T-helper cells) correlated best with increased non-relapse mortality (65). Nelson et al. prospectively measured the levels of multiple GVHD biomarkers at days 7, 14, 21, and 30 after the HSCT. They report that, although Reg3alpha was elevated at certain time points in patients who developed acute GVHD, this did not reach a statistically significant level and was not predictive for other endpoints, including the occurrence of severe GVHD, reduced overall survival, and increased non-relapse mortality (49). It can be argued that all these studies had a relatively small sample size and were underpowered to detect the prognostic value of all relevant biomarkers.

As a conclusion to this chapter, I would like to note that the levels of biomarkers (Reg3alpha and ST2) are routinely being measured at the time of GVHD onset at the department of allogeneic stem-cell transplantation in Regensburg, and these measurements serve as one additional piece of information in the complex decision-making algorithm in patients presenting with newly developed gastrointestinal symptoms. Further larger prospective studies are needed to determine the definitive place of GVHD biomarkers in everyday clinical practice, especially regarding the optimal timing of their assessment. The international MAGIC consortium is currently carrying out one such study, and the University Hospital Regensburg is coordinating the German centers.

4.3. Predictive value of the EASIX score

The Endothelial Activation and Stress Index (EASIX) score, retrospectively analyzed in this study in 108 patients without clinical symptoms of acute GVHD in an early post-allogeneic-HSCT setting, correlated with increased non-relapse mortality at 12 and 24 months after the transplantation. These findings should not be surprising as they resonate well with other recent studies that have confirmed and broadened the EASIX score’s predictive potential in allogeneic-HSCT patients (60,66,67). EASIX was initially developed in a post–HSCT setting as Luft et al. showed that patients with higher scores measured at the onset of acute GVHD had increased non-relapse mortality and reduced overall survival (59).

The EASIX score – calculated using the values of serum lactate dehydrogenase, serum creatinine, and thrombocytes – is at least partly driven by endothelial vulnerability, its dysfunction, and activation (60). Endothelial stress markers have previously been shown to be independent predictors of non-relapse mortality, supporting the hypothesis that endothelial damage plays a significant role in the pathophysiology of major complications of allogeneic HSCT (68). A growing amount of evidence suggests that endothelial damage predates the HSCT and its preparative – conditioning treatment itself. In a recent study by Luft and his group, EASIX was measured prior to the conditioning therapy in more than 2000 patients from five independent cohorts (60). Increased EASIX-score values prior to allogeneic HSCT significantly correlated with reduced overall survival and increased non-relapse mortality (the hazard ratios were 1.14 and 1.23 respectively). Furthermore, although not statistically significant in uni- and multivariable analyses, higher EASIX values tended to be associated with higher risk of Grade III–IV acute GVHD (60).

Another group recently reported very similar findings in a single-center study (528 patients), thus confirming the predictive potential of the EASIX score measured prior to the transplantation (66). The EASIX score was compared with other commonly used scoring systems in allogeneic-HSCT settings and was demonstrated to be among the strongest predictors of non-relapse mortality (66). Authors report that the score demonstrated higher predictive value in patients who later received myeloablative conditioning, and they argue that these patients could be more susceptible to developing endothelial dysfunction (66). Another significant complication of the allogeneic HSCT was recently linked with the higher values of the EASIX. Varma et al. show that patients with elevated EASIX score at the time of admission were significantly more likely to experience fluid overload during their hospital stay (67). They speculate that this could be due to increased capillary permeability in inflammatory conditions related to the endothelial damage.

It must be noted, however, that despite the promising results in both studies published by Luft and his research team (59,60), the hazard ratios were relatively low for both overall survival and non-relapse mortality (between 1.1 and 1.4 per log2 increase). This suggests that the EASIX score cannot account for all the complex pathophysiological changes occurring during the acute GVHD, and that accurate

individualized prediction remains suboptimal. Prognostic systems like EASIX may be useful for risk stratification, but individual prediction remains challenging in everyday clinical practice, necessitating caution when making decisions based on the results of these tools.

The EASIX score could, interestingly, become a broadly applicable predictive tool beyond the scope of HSCT (69) as endothelial dysfunction and angiogenesis play an important role in a variety of hematologic malignancies (69). The score proved to be a reliable predictor for overall survival in patients with multiple myeloma at the time of the diagnosis (69) and of low-risk myelodysplastic syndromes (70). These interesting potential applications of the EASIX score remain, however, beyond the scope of this dissertation.

4.4. Limitations, strengths, and future perspectives

There are several limitations to this study. Due to its retrospective design, based on data from a single center, the findings should be interpreted with caution. The number of patients included was limited, especially in regard to patients showing no symptoms. The predictive value of early gastrointestinal-screening biopsies should be further evaluated in a larger cohort, as a part of a multicenter prospective study, with a special focus on asymptomatic patients.

It should be acknowledged here that histological interpretation of gastrointestinal biopsies can be difficult, as many of the changes occurring as part of gastrointestinal GVHD show overlapping features with a multitude of other causes, such as side effects of the conditioning regimen, or various infections. Improving on histological findings could resolve such diagnostic dilemmas, particularly with the introduction of specialized procedures, such as the addition of Paneth-cell counts, or more sensitive caspase staining for the recognition of mucosal apoptosis. These interventions have their own limitations and would require more resources and additional experience, which are not always readily available in all centers.

The optimal timing of the screening biopsy remains unclear, as it should ideally be performed before the onset of GVHD. Early biopsies, performed within the first two weeks following the allogeneic HSCT, lead to differential diagnostic dilemmas,

making the interpretation challenging. Postponing the screening biopsy, however, leads to the delayed recognition of patients at high risk, limiting the likelihood of an early therapeutic intervention. Performing multiple, serial, gastrointestinal biopsies could be considered as a preferrable course of action and it would provide additional valuable information regarding this question. Due to the invasive nature of the procedure, this kind of approach seems impractical.

One of the strengths of this study is that it adds another predictive approach to early recognition of high-risk patients, improving our diagnostic resources. As the time of analysis was prior to the onset of the GVHD symptoms, the information gained is likely to be actionable. The early findings reported in this study seem to be promising. Combining multiple GVHD scoring systems (such as histological grading, values of different biomarkers, or clinical scores such as EASIX) has the potential to further improve predictive value, and it should be examined in a follow-up, prospective study.

5. Conclusion

In conclusion, early histological changes in the gut mucosa provide valuable information in the post-allogeneic-HSCT setting and histological assessment should be an integral part of the comprehensive diagnostic approach taken to patients presenting with any gastrointestinal symptoms. This study supports the idea of performing early-screening biopsies of gastrointestinal mucosa, especially in patients at high risk of developing GVHD, as the data implies that early histological changes predate the development of the gastrointestinal symptoms in a significant subset of patients.

Prognostic systems like EASIX and biomarker-based scores are useful for risk stratification, but individual prediction remains challenging in everyday clinical practice as the relatively low prevalence of severe acute GVHD limits the positive predictive value of these tools. Specifically, this study does not support the concept that biomarkers can substitute early biopsies.

Further larger prospective clinical trials are needed, and optimal timing of an early-screening biopsy remains to be debated.

6. Appendix

6.1. List of tables

6.2. List of figures

6.3. List of abbreviations

aGVHD	Acute graft-versus-host disease
AMPs	Antimicrobial peptides
APCs	Antigen-presenting cells
ASBMT	American Society of Blood and Marrow Transplant
BMT	Bone-marrow transplantation
BSA	Body surface area
cGVHD	Chronic graft-versus-host disease
CIBMTR	Center for International Blood and Marrow Transplant Research
CMV	Cytomegalovirus
DAMPs	Damage-associated molecular patterns
DKFZ	German Cancer Research Center
dL	Deciliter
EASIX	The Endothelial Activation and Stress Index
GI	Gastrointestinal
GVHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem-cell transplantation
IgA	Immunoglobulin A
IL-1	interleukin 1
LDH	Lactate dehydrogenase

MAGIC	Mount Sinai Acute GVHD International Consortium
mg	Milligram
ml	Milliliter
mTOR	Mammalian target of rapamycin
ng	Nanogram
NIH	National Institute of Health
NRM	Non-relapse mortality
OS	Overall survival
PAMPs	Pathogen-associated molecular patterns
PBSCT	Peripheral blood stem-cell transplantation
Reg3alpha	Regenerating islet-derived protein 3 alpha
RIC	Reduced-intensity chemotherapy
ST2	Suppressor of tumorigenicity 2
TIM3	T-cell immunoglobulin and mucin-domain containing-3
TNF-α	tumor necrosis factor alpha
TNFR1	Tumor necrosis factor receptor 1

7. Literature

1. Mathé G, Amiel JL. A Haematologist's Approach to Modern Grafting Problems. Br Med J. 1964 Aug 29;2(5408):527–30.
2. 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 J Am Soc Blood Marrow Transplant. 2005 Dec;11(12):945–56.
3. 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 J Am Soc Blood Marrow Transplant. 2015 Mar;21(3):389-401.e1.
4. 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 Nov;53(11):1401–15.
5. 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 Jul;158(1):30–45.
6. Ferrara JLM, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. Int J Hematol. 2003 Oct;78(3):181–7.
7. Reddy P, Ferrara JLM. Immunobiology of acute graft-versus-host disease. Blood Rev. 2003 Dec;17(4):187–94.
8. 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 [Internet]. 7th ed. Cham (CH): Springer; 2019 [cited 2021 Sep 27]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK553993/

9. Ferrara JLM. Cellular and cytokine effectors of acute graft versus host disease. Int J Hematol. 2002 Aug;76 Suppl 1:195–8.

10. Jaksch M, Mattsson J. The pathophysiology of acute graft-versus-host disease. Scand J Immunol. 2005 May;61(5):398–409.

11. Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet Lond Engl. 2009 May 2;373(9674):1550–61.

12. Holler E, Kolb HJ, Möller A, Kempeni J, Liesenfeld S, Pechumer H, et al. Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. Blood. 1990 Feb 15;75(4):1011–6.

13. 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 Oct;18(4):295–304.

14. 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 Jun;15(6):825–8.

15. 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 J Am Soc Blood Marrow Transplant. 2016 Jan;22(1):4–10.

16. Elphick DA, Mahida YR. Paneth cells: their role in innate immunity and inflammatory disease. Gut. 2005 Dec;54(12):1802–9.

17. van der Waaij LA, Limburg PC, Mesander G, van der Waaij D. In vivo IgA coating of anaerobic bacteria in human faeces. Gut. 1996 Mar;38(3):348–54.

18. Ferrara JLM, Chaudhry MS. GVHD: biology matters. Blood Adv. 2018 Nov 27;2(22):3411–7.

19. Holler E, Landfried K, Meier J, Hausmann M, Rogler G. The role of bacteria and pattern recognition receptors in GVHD. Int J Inflamm. 2010 Oct 31;2010:814326.

20. 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 J Am Soc Blood Marrow Transplant. 2014 May;20(5):640–5.

21. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med. 2016 May 18;8(339):339ra71.

22. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota Disruption Induced by Early Use of Broad-Spectrum Antibiotics Is an Independent Risk Factor of Outcome after Allogeneic Stem Cell Transplantation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2017 May;23(5):845–52.

23. Taur Y, Jenq RR, Perales M-A, 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 Aug 14;124(7):1174–82.

24. Levine JE, Huber E, Hammer STG, 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 Aug 22;122(8):1505–9.

25. Holler E, Rogler G, Brenmoehl J, Hahn J, Greinix H, Dickinson AM, et al. The role of genetic variants of NOD2/CARD15, a receptor of the innate immune system, in GvHD and complications following related and unrelated donor haematopoietic stem cell transplantation. Int J Immunogenet. 2008 Aug;35(4–5):381–4.

26. McDonald GB. How I treat acute graft-versus-host disease of the gastrointestinal tract and the liver. Blood. 2016 Mar 24;127(12):1544–50.

27. Naymagon S, Naymagon L, Wong S-Y, Ko HM, Renteria A, Levine J, et al. Acute graft-versus-host disease of the gut: considerations for the gastroenterologist. Nat Rev Gastroenterol Hepatol. 2017 Dec;14(12):711–26.

28. Van Lint MT, Uderzo C, Locasciulli A, Majolino I, Scimé R, Locatelli F, et al. Early Treatment of Acute Graft-Versus-Host Disease With High- or Low-Dose 6-Methylprednisolone: A Multicenter Randomized Trial From the Italian Group for Bone Marrow Transplantation. Blood. 1998 Oct 1;92(7):2288–93.

29. Bertz H, Afting M, Kreisel W, Duffner U, Greinwald R, Finke J. Feasibility and response to budesonide as topical corticosteroid therapy for acute intestinal GVHD. Bone Marrow Transplant. 1999 Dec;24(11):1185–9.

30. 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 J Am Soc Blood Marrow Transplant. 2009 Apr;15(4):10.1016/j.bbmt.2008.12.487.

31. Weisdorf D, Haake R, Blazar B, Miller W, McGlave P, Ramsay N, et al. Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. Blood. 1990 Feb 15;75(4):1024–30.

32. MacMillan ML, Weisdorf DJ, Wagner JE, DeFor TE, Burns LJ, Ramsay NKC, et al. Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2002;8(7):387–94.

33. Westin JR, Saliba RM, De Lima M, Alousi A, Hosing C, Qazilbash MH, et al. Steroid-Refractory Acute GVHD: Predictors and Outcomes. Adv Hematol. 2011;2011:601953.

34. Wolff D, Schleuning M, von Harsdorf S, Bacher U, Gerbitz A, Stadler M, et al. Consensus Conference on Clinical Practice in Chronic GVHD: Second-Line Treatment of Chronic Graft-versus-Host Disease. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2011 Jan;17(1):1–17.

35. Myerson D, Steinbach G, Gooley TA, Shulman HM. Graft-versus-Host Disease of the Gut: A Histologic Activity Grading System and Validation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2017 Sep;23(9):1573–9.

36. Iqbal N, Salzman D, Lazenby AJ, Wilcox CM. Diagnosis of gastrointestinal graft-versus-host disease. Am J Gastroenterol. 2000 Nov;95(11):3034–8.

37. 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 Sep;38(5):371–6.

38. Ross WA, Ghosh S, Dekovich AA, Liu S, Ayers GD, Cleary KR, et al. Endoscopic biopsy diagnosis of acute gastrointestinal graft-versus-host disease: rectosigmoid biopsies are more sensitive than upper gastrointestinal biopsies. Am J Gastroenterol. 2008 Apr;103(4):982–9.

39. Narkhede M, Rybicki L, Abounader D, Bolwell B, Dean R, Gerds AT, et al. The association of histologic grade with acute graft-versus-host disease response and outcomes. Am J Hematol. 2017 Jul;92(7):683–8.

40. Washington K, Jagasia M. Pathology of graft-versus-host disease in the gastrointestinal tract. Hum Pathol. 2009 Jul;40(7):909–17.

41. Salomao M, Dorritie K, Mapara MY, Sepulveda A. Histopathology of Graft-vs-Host Disease of Gastrointestinal Tract and Liver: An Update. Am J Clin Pathol. 2016 May;145(5):591–603.

42. 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 Dec;6(4):367–71.

43. 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 J Am Soc Blood Marrow Transplant. 2015 Apr;21(4):589–603.

44. Rowlings PA, Przepiorka D, Klein JP, Gale RP, Passweg JR, Henslee-Downey PJ, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol. 1997 Jun;97(4):855–64.

45. MacMillan ML, DeFor TE, Weisdorf DJ. What predicts high risk acute graft-versus-host disease (GVHD) at onset?: identification of those at highest risk by a novel acute GVHD risk score. Br J Haematol. 2012 Jun;157(6):732–41.

46. MacMillan ML, Robin M, Harris AC, DeFor TE, Martin PJ, Alousi A, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2015 Apr;21(4):761–7.

47. MacMillan ML, DeFor TE, Holtan SG, Rashidi A, Blazar BR, Weisdorf DJ. Validation of Minnesota acute graft-versus-host disease Risk Score. Haematologica. 2020;105(2):519–24.

48. Hartwell MJ, Özbek U, Holler E, Renteria AS, Major-Monfried H, Reddy P, et al. An early-biomarker algorithm predicts lethal graft-versus-host disease and survival. JCI Insight. 2(3):e89798.

49. Nelson RP, Khawaja MR, Perkins SM, Elmore L, Mumaw CL, Orschell C, et al. Prognostic biomarkers for acute graft-versus-host disease risk after cyclophosphamide-fludarabine nonmyeloablative allotransplantation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2014 Nov;20(11):1861–4.

50. Ferrara JLM, 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 Dec 15;118(25):6702–8.

51. McDonald GB, Tabellini L, Storer BE, Lawler RL, Martin PJ, Hansen JA. Plasma biomarkers of acute GVHD and nonrelapse mortality: predictive value of measurements before GVHD onset and treatment. Blood. 2015 Jul 2;126(1):113–20.

52. Levine JE, Braun TM, Harris AC, Holler E, Taylor A, Miller H, et al. A prognostic score for acute graft-versus-host disease based on biomarkers: a multicentre study. Lancet Haematol. 2015 Jan;2(1):e21-29.

53. Rezvani AR, Storer BE, Storb RF, Mielcarek M, Maloney DG, Sandmaier BM, et al. Decreased serum albumin as a biomarker for severe acute graft-vs.-host disease after reduced-intensity allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2011 Nov;17(11):1594–601.

54. Rodriguez-Otero P, Porcher R, Peffault de Latour R, Contreras M, Bouhnik Y, Xhaard A, et al. Fecal calprotectin and alpha-1 antitrypsin predict severity and response to corticosteroids in gastrointestinal graft-versus-host disease. Blood. 2012 Jun 14;119(24):5909–17.

55. Major-Monfried H, Renteria AS, Pawarode A, Reddy P, Ayuk F, Holler E, et al. MAGIC biomarkers predict long-term outcomes for steroid-resistant acute GVHD. Blood. 2018 Jun 21;131(25):2846–55.

56. Ali AM, DiPersio JF, Schroeder MA. The Role of Biomarkers in the Diagnosis and Risk Stratification of Acute Graft-versus-Host Disease: A Systematic Review. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2016 Sep;22(9):1552–64.

57. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science. 2006 Aug 25;313(5790):1126–30.

58. Zhao D, Kim Y-H, Jeong S, Greenson JK, Chaudhry MS, Hoepting M, et al. Survival signal REG3α prevents crypt apoptosis to control acute gastrointestinal graft-versus-host disease. J Clin Invest. 2018 Nov 1;128(11):4970–9.

59. Luft T, Benner A, Jodele S, Dandoy CE, Storb R, Gooley T, et al. EASIX in patients with acute graft-versus-host disease: a retrospective cohort analysis. Lancet Haematol. 2017 Sep;4(9):e414–23.

60. Luft T, Benner A, Terzer T, Jodele S, Dandoy CE, Storb R, et al. EASIX and mortality after allogeneic stem cell transplantation. Bone Marrow Transplant. 2020 Mar;55(3):553–61.

61. 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 Oct;82(10):881–6.

62. Abraham J, Janin A, Gornet J-M, Peffault de Latour R, Robin M, Xhaard A, et al. Clinical severity scores in gastrointestinal graft-versus-host disease. Transplantation. 2014 May 15;97(9):965–71.

63. 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 Nov;41(11):1483–90.

64. Weber D, Frauenschläger K, Ghimire S, Peter K, Panzer I, Hiergeist A, et al. The association between acute graft-versus-host disease and antimicrobial peptide expression in the gastrointestinal tract after allogeneic stem cell transplantation. PloS One. 2017;12(9):e0185265.

65. Balakrishnan B, Illangeswaran RSS, Rajamani BM, Pai AA, Raj IX, Paul DZ, et al. Prognostic plasma biomarkers of early complications and graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation. eJHaem. 2020;1(1):219–29.

66. Shouval R, Fein JA, Shouval A, Danylesko I, Shem-Tov N, Zlotnik M, et al. External validation and comparison of multiple prognostic scores in allogeneic hematopoietic stem cell transplantation. Blood Adv. 2019 Jun 20;3(12):1881–90.

67. Varma A, Rondon G, Srour SA, Chen J, Ledesma C, Champlin RE, et al. Endothelial Activation and Stress Index (EASIX) at Admission Predicts Fluid Overload in Recipients of Allogeneic Stem Cell Transplantation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2020 May;26(5):1013–20.

68. Dietrich S, Falk CS, Benner A, Karamustafa S, Hahn E, Andrulis M, et al. Endothelial vulnerability and endothelial damage are associated with risk of graft-versus-host disease and response to steroid treatment. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2013 Jan;19(1):22–7.

69. Song G-Y, Jung S-H, Kim K, Kim SJ, Yoon SE, Lee HS, et al. Endothelial activation and stress index (EASIX) is a reliable predictor for overall survival in patients with multiple myeloma. BMC Cancer. 2020 Aug 24;20(1):803.

70. Merz A, Germing U, Kobbe G, Kaivers J, Jauch A, Radujkovic A, et al. EASIX for prediction of survival in lower-risk myelodysplastic syndromes. Blood Cancer J. 2019 Nov 11;9(11):85.

8. Acknowledgments

I would like to thank Prof. Dr. Ernst Holler for suggesting the topic to me, for his advice and help throughout the entire process. His guidance, knowledge and comments helped me immensely with my work.

Special thanks are due to the team of the *KMT-Ambulanz* from the University Hospital in Regensburg for providing me with help (and chocolate) during my archival research.

I would like to thank Dr. Mathias Pantke and Dr. Andrew Hodges for proofreading the thesis and for providing helpful comments.

Finally, I would like to thank my wife, Čarna Brković, for her endless support and love. And I would like to thank my son, Ivo Martinović, for providing me with all the joy only a toddler can provide.

Dragi Milice, Drago, Ana i Milane, hvala vam, volim vas.

9. Curriculum vitae

Education

2003 – 2010	School of Medicine, University of Belgrade, Serbia. GPA 9.37 (5.00-10.00)

Employment

2020 onwards	Department of Hematology and Medical Oncology Unit of Allogeneic Stem Cell Transplantation University Medical Center Göttingen, Germany
2016 – 2020	Internal Medicine III Department (Hematology and Oncology) University Hospital Regensburg, Germany
2012 – 2015	Oncology and Radiotherapy Clinic, Chemotherapy Department, Clinical Center of Montenegro
2011 – 2012	Primary Healthcare Unit, Podgorica

Development Courses

2016	European Society for Medical Oncology (ESMO) Preceptorship on Gastric Cancer: “Multidisciplinary management, standards of care, therapeutic targets and future Perspectives”, 10-11 June. Prague, Czech Republic
2015	European Society for Medical Oncology (ESMO) Preceptorship on Gastric Cancer: “Multidisciplinary management, standards of care, therapeutic targets and future Perspectives”, 4-5 September. Brussels, Belgium
2015	Training in Monitoring of Non-Infectious Diseases and Related Health Inequalities – Defining the Minimal Set of Indicators, Institute of Public Health of Montenegro, Ministry of Health of Montenegro, and European Commission. Podgorica, Montenegro
2014	Observership Program, Palliative Care Unit, Clinic for Oncology, 20-31 October 2014. Mentor: Dr D. Jahn-Kuch. Medical University of Graz, Austria

Activities

2014 onwards	ESMO member
2015	Teacher of the exchange students of medicine in Montenegro, SCOPE program, *International Federation of Medical Students' Associations* (IFMSA)
2014 and 2015	Volunteering: Clinical Breast Examinations, Breast Cancer Awareness Campaign, NGO "Prava priča" and Clinical Centre Montenegro, October

Publications

2020	Kattner AS, Holler E, Holler B, Klobuch S, Weber D, Martinovic D, Edinger M, Herr W, Wolff D. IL6-receptor antibody tocilizumab as salvage therapy in severe chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. Ann Hematol. 2020 Apr;99(4)847-853. doi: 10.1007/s00277-020-03968-w. Epub 2020 Feb 21.
2015	Todorović V, Martinović D. "Skin Toxicity of Targeted Therapy: Vemurafenib, First Experiences from Montenegro". *Sanamed: Medical Journal of Doctors Novi Pazar.* 2015;10(2):109-14.