Diploma thesis

Impact of hyperglycemia on the outcome of graft-versus-host disease: a retrospective analysis

submitted by

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Zusammenfassung

Einführung: Zu den häufigsten Komplikationen einer allogenen Stammzelltransplantation gehört die Graft-versus-host disease (GvHD). Da die Standard-Erstlinientherapie auf Glucocorticoiden basiert, entwickelt sich bei PatientInnen mit GvHD häufig eine Steroid-induzierte Hyperglykämie. Trotz der Häufigkeit des Auftretens ist wenig über den Einfluss der Hyperglykämie auf das Outcome dieser PatientInnen bekannt.

Methodik: Wir führten eine retrospektive Analyse mit 102 PatientInnen durch, welche eine systemische Glucocorticoidtherapie wegen akuter oder chronischer GvHD erhielten. Nur bei einem Patienten war ein Diabetes mellitus vorbekannt. Wir untersuchten den Einfluss der Hyperglykämie auf das Gesamtüberleben bzw. die behandlungsbezogene Sterberate der PatientInnen. Regelmäßige Messungen der Blutglucose wurden für jeden einzelnen während der Glucocorticoidtherapie aufgezeichnet. Zusätzlich wurden Parameter bezüglich der Transplantation sowie GvHD eingeholt (Grunderkrankung, Zeitpunkt des Auftretens der GvHD, Anzahl der betroffenen Organe, Ansprechen auf Glucocorticoide, Insulintherapie und (Pilz)-Infektionen). Die PatientInnen wurden gemäß ihrer Blutglucose in Quartilen eingeteilt. Der mediane, mittlere, maximale und minimale Glucosewert wurde für diese Analysen herangezogen.

Ergebnisse: Während die mediane Überlebenszeit bei PatientInnen der ersten Quartile nicht erreicht wurde, da deutlich weniger als die Hälfte dieser PatientInnen verstorben ist, waren höhere mediane Blutglucose-Werte mit einem verminderten medianen Überleben von 19, 8 und 8 Monaten in den Quartilen 2, 3 und 4 assoziiert ($p = 0.0091$). Diese Assoziation zeigte sich auch für die mittleren und maximalen Blutzuckerwerte. Zusätzlich zeigten PatientInnen, bei denen eine Insulin-Behandlung notwendig war ($n = 49$), ein verkürztes medianes Überleben (8 versus 38 Monate, $p = 0.0077$). Während die Relaps-bedingte Sterberate nicht beeinflusst wurde, war eine Hyperglykämie mit einer erhöhten behandlungsbezogenen Mortalität assoziiert. Insbesondere hatten PatientInnen, bei denen eine invasive Pilzinfektion auftrat, ein sehr schlechtes Outcome (mediane Überlebensrate von 5 versus 24 Monate, $p = 0.0006$). Schließlich wurde in einer multivariaten Analyse das Auftreten einer Hyperglykämie als bedeutsamster Risikofaktor identifiziert.

Schlussfolgerung: In dieser retrospektiven Analyse stellten wir einen nachteiligen Einfluss der Glucocorticoid-induzierten Hyperglykämie auf das Outcome von PatientInnen mit GvHD fest. Prospektive Studien, die eine strenge glykämische Kontrolle prüfen, werden in diesem klinischen Setting dringend benötigt.

Abstract

Introduction: Graft-versus-host disease (GvHD) remains one of the most severe complications after allogeneic hematopoietic stem cell tranplantation (HSCT). Since the current standard first-line treatment is based on high-dose glucocorticoid therapy, steroid-induced hyperglycemia develops frequently in patients with GvHD. However, little is known about the impact of hyperglycemia on outcome of GvHD.

Methods: We performed a retrospective analysis of 102 patients who received systemic glucocorticoid therapy due to acute or chronic GvHD and investigated the impact of hyperglycemia on overall survival (OS) and transplant-related mortality (TRM) in these patients. For each subject, regular blood glucose measurements during glucocorticoid therapy were recorded. In addition, transplant- as well as GvHD-related parameters, such as underlying disease, time to onset of GvHD, number of affected organs, response to glucocorticoids, insulin therapy and (fungal)-infectious complications were obtained. For analysis, the median, mean, maximum and minimum glucose levels were taken and patients were divided into quartiles according to their glucose levels.

Results: With a median of 42 (range 4-249) blood glucose measurements, 25 patients each were assigned to quartile 1 and 2 and 26 patients to quartile 3 and 4. While median OS was not reached in quartile 1, due to low numbers of death, increasing median blood glucose levels were associated with decreasing median OS of 19, 8 and 8 months for quartiles 2, 3 and 4, respectively ($p = 0.0091$). Similar results were obtained when mean and maximal glucose levels were used. In addition, patients who needed insulin treatment ($n = 49$) had a shorter median OS (8 versus 38 months, $p = 0.0077$). While the numbers of death due to relapse were not affected, high blood glucose values were associated with increased TRM rates. In particular, patients with invasive fungal infections (IFI) had a poor outcome (median OS of 5 months versus 24 months, $p = 0.0006$). In a multivariate analysis, including age at diagnosis, gender, organs affected by GvHD, BMI, donor type, HLA-matching and gender-matching, hyperglycemia remained the most prominent risk factor for short OS.

Conclusion: In this retrospective analysis we observed an adverse impact of glucocorticoid-induced hyperglycemia on outcome in patients with GvDH. Prospective trials testing a stringent glycemic control are therefore urgently needed in this clinical setting.

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Abbreviations

ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
APC	Antigen-presenting cell
ATG	Antithymocyte globulin
BMI	Body mass index
CAP	Community-acquired pneumonia
cGvHD	Chronic GvHD
CI	Confidence interval
dl	Deciliter
ECP	Extracorporeal photopheresis
G-CSF	Granulocyte-colony stimulating factor
GvHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HR	Hazard ratio
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
IFI	Invasive fungal infection
IFN	Interferon
IU	International unit
L	Liter
LPS	Lipopolysaccharide
MDS	Myelodysplastic syndrome
MEDOCS	Medical Documentation and Communication System
mg	Milligram
ml	Milliliter
MM	Multiple myeloma
mTOR	Mammalian target of rapamycin
NIH	National Institutes of Health
NRM	Non-relapse mortality
OR	Odds ratio
OS	Overall survival

TGC	Tight glycemic control
TNF	Tumor necrosis factor
TRM	Transplant-related mortality
UTI	Urinary tract infection

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1 Introduction

More than 50 years ago, E.D. Thomas and his colleagues first described the infusion of bone marrow cells into a patient with end-stage leukemia. After the identification of human leukocyte antigens (HLA), allogeneic hematopoietic stem cell transplantation (HSCT) became feasible in the early 1970s. Due to scientific and clinical progress in the last decades, the use of HSCT has become a widely used standard treatment procedure in patients with hematological malignancies and other diseases. (1,2)

Although HSCT possesses a curative potential for many hematological patients, its success is significantly limited by the occurrence of acute and chronic graft-versus-host disease (GvHD). GvHD is a systemic immune-mediated disorder, where donor-derived T-cells attack cells and tissues of the recipient. It seriously affects the quality of life but also survival of patients after HSCT. (1,2) Since glucocorticoids are the established first-line treatment of GvHD, disturbances of glucose metabolism are a frequent side effect. However, whether or not hyperglycemia may affect morbidity and mortality in patients suffering from GvHD is yet largely unknown. As a consequence, there is also significant clinical uncertainty on how strict glucose should be controlled in these subjects.

In this diploma thesis, we retrospectively analyzed the outcome of patients who required systemic glucocorticoids due to GvHD after HSCT at the Division of Hematology, at the Medical University of Graz.

1.1 *Hematopoietic stem cell transplantation (HSCT)*

HSCT is an important and well-established curative treatment for a variety of malignant and nonmalignant hematological diseases. (1-3) This procedure includes chemotherapy with or without total body irradiation and is followed by infusion of hematopoietic stem cells (HSC), which then home to the bone marrow and start replenishing blood cells. While chemotherapy and total body irradiation aim at eradication of malignant cells of the underlying disease (most commonly acute leukemia), infusion of HSC is needed to rebuild patient's immune and hematopoietic system. (1,2) HSCT can be categorized as autologous

(when the HSC recipient receives his own stem cells), as allogeneic (HSC are obtained from an HLA-matched donor) or syngeneic (stem cells from an identical twin). (1)

1.1.1 Indications for hematopoietic stem cell transplantation

More than 37 000 HSCT were performed in Europe in 2012. The majority of patients (about 22 500) received an autologous HSCT, with multiple myeloma, relapsed Non-Hodgkin's lymphoma and relapsed Hodgkin's disease among the most common indications. About 15 000 patients underwent allogeneic HSCT in Europe in 2012. Among these, most patients were affected with acute leukemia. (1,4,5) A summary of diseases commonly treated with HSCT is listed below.

Table 1: Diseases commonly treated with HSCT

Autologous transplantation	Allogeneic transplantation
Multiple myeloma	Acute myeloid leukemia
Non Hodgkin's lymphoma	Acute lymphoblastic leukemia
Hodgkin's disease	Chronic myeloid leukemia
Acute myeloid leukemia	Myelodysplastic syndromes
Neuroblastoma	Myeloproliferative disorders
Ovarian cancer	Non-Hodgkin's lymphoma
Germ-cell tumors	Hodgkin's disease
Autoimmune disorders	Chronic lymphocytic leukemia
Amyloidosis	Multiple myeloma
	Aplastic anemia
	Fanconi's anemia
	Paroxysmal nocturnal hemoglobinuria
	Thalassemia major
	Sickle cell anemia
	Wiskot-Aldrich syndrome

adapted from Copelan EA; The New England Journal of Medicine 2006 (1)

1.1.2 Sources of stem cells

Direct collection from the bone marrow was the first source of HSC. More recently, peripheral blood and umbilical cord blood have emerged as important stem cell sources in clinical practice. (1,2)

1.1.2.1 Bone marrow

For bone marrow transplantation, HSC are harvested from the posterior iliac crest. However, due to the requirement of general anesthesia, bone marrow is not frequently used anymore as a stem cell source. (1)

1.1.2.2 Peripheral blood

Peripheral blood has widely replaced bone marrow for autologous as well as allogeneic transplant procedures. (1,2) Mobilization of HSC to peripheral blood can be induced by using granulocyte colony-stimulating factor (G-CSF). G-CSF induces neutrophil activation and the secretion of proteases in the bone marrow. This process results in egress of HSC to the peripheral blood. As an additional or alternative agent for hematopoietic stem cell mobilization, the CXC chemokine receptor 4 (CXCR 4) inhibitor plerixafor is clinically available. CXCR4 is expressed on HSC and mediates their homing, the HSC niche in the bone marrow. (1,2) After mobilization of HSC to the peripheral blood, HSC can be harvested by leukapheresis. One advantage of peripheral blood as stem cell source is the more rapid hematopoietic reconstitution after HSCT. In general, 2-8x10^6 CD34+ cells per kg bodyweight are needed. (1)

1.1.2.3 Umbilical cord blood (UCB)

Blood from the umbilical cord contains a high number of HSC. (1) Thus, UCB stem cells are cryopreserved and stored in cord blood banks. Its immediate availability and the less stringent requirements for HLA-matching of the donor and the recipient represent advantage for HSCT recipients. However, due to low HSC numbers transfused in correlation to the bodyweight (especially in adult recipients), UCB-HSCT is characterized by an in-

creased risk for engraftment failure. (6,7) Further information on UCB is given in chapter 1.1.3.2.

1.1.3 Graft types

1.1.3.1 Autologous hematopoietic stem cell transplantation

In the case of autologous stem cell transplantation, the patient's HSC are isolated prior to the conditioning procedure (see 1.1.4) and stored in liquid nitrogen. After myeloablation with chemotherapy and/or irradiation, the collected HSC are thawed and reinfused to rescue hematopoiesis. Transplantation of autologous HSC is routinely used in patients with multiple myeloma and relapsed lymphoma. (1,2)

1.1.3.2 Allogeneic hematopoietic stem cell transplantation

In case of allogeneic HSCT, HSC are obtained from a relative (most commonly an HLA-matched sibling) or an unrelated person who has been identified from a world-wide registry of persons willing to donate bone marrow of peripheral blood HSC. This type of stem cell transplantation has become available through the discovery of HLA-typing. The HLA-system or major histocompatibility complex (MHC) is a group of molecules containing more than 200 genes, which are central to the function of the immune system and are categorized into Class I HLA (A, B, C) proteins and Class II HLA (DR, DQ, DP) proteins. (8-11) While class I proteins are present on the surface of almost all cells, class II are only expressed on hematopoietic cells. (11,12). "The primary role of HLA molecules is to present peptide to T-cells, enabling them to recognize and eliminate foreign particles present in an individual." (13) "The HLA system displays extensive polymorphisms most likely due to the need for the immune system to keep up with and control infectious pathogens." (13) Virtually no human being has the same composition of MHC molecules (besides monozygotic twins). Despite this massive diversity, it is possible to compare MHC molecules and identify compatible donors. Currently, in most patients matching is performed for both alleles on six HLA loci (HLA-A, -B, -C, -DR, -DQ, -DP). (13) The higher the diversity is between donors and recipients major histocompatibility antigens, the higher is

the risk for development of GvHD. (14,15) Since about 70% of patients lack a related donor and can't find a suitable unrelated donor, alternative graft sources, such as UCB and haplo-identical donors have recently emerged. (1,16)

A haplo-identical donor may be the patient's parent, sibling or child, who is only partially HLA-compatible with the recipient. The advantage of having a haplo-identical donor is its rapid availability. However, a variety of complications including GvHD, graft failure and relapse may occur. (1) Haplo-identical siblings with noninherited maternal antigens are associated with better outcomes in GvHD and reduced transplant-related mortality (TRM) rates as haplo-identical siblings with noninherited paternal antigens. (17) Some recipients, particularly people from minorities will not have an HLA-compatible donor available, because they are underrepresented in the international stem cell donor registry. In such situations, HSC from UCB can be an option. The number of transplantations performed with UCB has grown over the last 2 decades. (1,18) "The exact reason for the reduced incidence of GvHD remains to be determined, but a commonly accepted hypothesis is that cord blood-derived hematopoietic stem cells are more immunologically naive as their bone marrow-derived counterparts." (2) Thus, these transplants have the advantage of low numbers of immunoreactive T-cells. However, due to the low numbers of CD34+ HSC in UCB, delayed engraftment is the most important drawback of UCB HSCT, particular in adults. (18) Furthermore, the risk of infections is increased due to slow immunologic recovery. (1) Several methods in decreasing TRM are tested in clinical trials. (18)

1.1.3.3 Syngeneic hematopoietic stem cell transplantation

The option of performing HSCT from a genetically identical twin is rare. Due to syngeneity, there is almost no risk of GvHD, but previous studies have demonstrated an increased risk of relapse. (19,20)

1.1.4 Conditioning regimen

While in the autologous setting the conditioning regimens only aim for eradicating all malignant cells before HSC rescue, chemotherapy with or without irradiation is needed to suppress the patient's bone marrow and immune system before allogeneic HSCT. Thus, conditioning treatment allows engraftment of the donor's HSC and prevents graft failure. It also aims for eradication of the malignant cells, but is also intended to trigger an antileukemic or antitumor immune response. (1)

The standard preparation regimen since the 1980s has been fractionated total body irradiation combined with the chemotherapeutic agent cyclophosphamide. High dose chemotherapy (cyclophosphamide combined with busulfan) without radiation also proved effective in treating acute myeloid leukemia. (1) Due to substantial toxicity, such myeloablative regimens can only be used in patients under 55 years without co-morbidities. However, within the last 15 years non-myeloablative or reduced-intensity conditioning (RIC) regimens have been developed, allowing the treatment of older patients up to the age of 75 years. Primarily immunosuppressive, in these cases it depends on the graft cells to eradicate the malignant cells. (21) Fludarabine combined with or without total body irradiation, busulfan and melphalan are common chemotherapeutics used with RIC regimens. (22) In addition to chemo- and radiation therapy, immunosuppressive drugs, such as antithymocyte globulin (ATG), cyclosporin A (CsA), methotrexate (MTX) and mycophenolate mofetil (MMF) are needed to achieve engraftment of donor cells and to mitigate the occurrence of (acute) GvHD. (15,22)

1.1.5 Complications of hematopoietic stem cell transplantation

Transplant-associated complications comprise early and delayed events. While early adverse effects occur within the first 100 days of HSCT and include mucositis, veno-occlusive disease, infections and acute GvHD, late complications are chronic GvHD, infertility and an increased frequency of secondary malignancies affecting the skin, oral mucosa, brain, thyroid gland and the bone. (1) Besides severe infections, GvHD remains the major cause of morbidity and mortality after HSCT. (12)

1.2 *Graft-versus-host disease (GvHD)*

GvHD remains a common and potentially life-threatening complication of allogeneic HSCT. It presents with a heterogeneous clinical picture involving various organs, such as skin, upper and lower gastrointestinal tract or liver. (11,23) GvHD manifests in 2 forms, acute and chronic. While acute GvHD develops within the first 100 days after stem cell transplantation, chronic GvHD manifests later. However, a clear distinction of these two forms is not always possible. (24-26) Therefore, the National Institutes of Health (NIH) classification also defines a late-onset acute GvHD (starting after day 100) as well as an overlap syndrome, which is defined by the co-occurrence of acute and chronic features at once. (27,28)

1.2.1 **Acute GvHD**

Despite prophylactic treatment with immunosuppressive agents, acute GvHD occurs in 20 % to 80 % of recipients after HSCT. (29) It has a significant impact on morbidity and mortality in HSCT recipients. (23) Risk factors for the development of this severe complication include older age of the patient or donor, intensity of the conditioning regimen, donor and recipient sex difference (especially a female donor and a male recipient) and the use of peripheral blood HSC rather than bone marrow. (30) Whenever possible, the clinical diagnosis of acute GvHD should be confirmed by biopsy of an affected end organ. GvHD severity is graded by the Glucksberg criteria. (see 1.2.4) Occurrence of severe GvHD is associated with worse 5-year overall survival (OS) rates after HSCT (25 % for grade III and only 5 % OS for grade IVa GvHD). (31)

1.2.2 **Chronic GvHD**

At least 30 % to 50 % of recipients of HSC from an HLA-matched sibling develop chronic GvHD, while these percentages can even be higher in recipients of grafts from an unrelated donor (60 % to 70 %). (32) This complication remains the leading cause of late TRM. (27) Age (patient, donor), histoincompatibility, origin of graft (peripheral blood HSC versus bone marrow), donor (related versus unrelated) and previous acute GvHD are known to be the most important risk factors for the occurrence of chronic GvHD. (33,34) This disease

has an enormously wide range of clinical manifestations (skin, nails, mouth, eyes, muscles, joints, female genitalia, gastrointestinal tract, liver, lungs, kidneys, heart and marrow). (11) The clinical features and symptoms of acute and chronic GvHD are summarized in Table 2.

1.2.3 Clinical features and symptoms of GvHD

Acute GvHD	Chronic GvHD
Skin: maculopapular rash **Upper gastrointestinal tract:** nausea anorexia **Lower gastrointestinal tract:** watery diarrhea severe abdominal pain bloody diarrhea or ileus **Liver:** hyperbilirubinemia	**Skin:** dyspigmentation alopecia lichen planus-like eruptions poikiloderma **Nails:** naildystrophy or loss **Mouth:** xerostermia ulcers lichen-type features **Eyes:** sicca syndrome ciatricial conjunctivitis **Muscles, fascia, joints:** fasciitis myositis joint stiffness from contractures **Female genitalia:** vaginal sclerosis ulcerations **Gastrointestinal tract:** anorexia weight loss esophageal web or strictures **Liver:** jaundice transaminitis **Lungs:** restrictive or obstructive defects on pulmonary function tests bronchiolitis obliterans pleural effusions **Kidneys:** nephrotic syndrome (rare) **Heart:** pericarditis **Marrow:** thrombocytopenia anemia

Table 2: Clinical features and symptoms of GvHD

adapted from Ferrara JL et al.; Lancet 2009 (11)

1.2.4 Staging and overall grading of GvHD

The (overall) severity of acute GvHD is determined by staging of the extent of clinical symptoms as well as distinct laboratory values related to the organs affected. Table 3 gives the exact definitions for staging of the distinct organs affected, while Table 4 summarizes the overall grading of GvHD according to the characteristics of all organs involved (see Table 2). These grading systems have proven very useful in predicting the outcome of acute GvHD (35,36).

Table 3: Staging of acute GvHD

Stage	Skin based on maculopapular rash	Liver based on serum bilirubin	Gastrointestinal tract based on quantity of diar-rhea
+	< 25 % of body surface area	34-50 µmol/L	> 500 < 1000 mL
++	25-50% of body surface area	51-102 µmol/L	> 1000 < 1500 mL
+++	Generalized erythroderma	103-255 µmol/L	> 1500 mL
++++	Generalized erythroderma with bulla formation	> 255 µmol/L	Severe abdominal pain with or without ileus

adapted from Apperley J et al.; The EBMT Handbook 2012
http://ebmtonline.forumservice.net/media/13/main.html (36)

Table 4: Overall grading of acute GvHD

Grade	Organ and stage involvement
I (mild)	Skin + to ++
II (moderate)	Skin + to +++ Gastrointestinal tract and/or liver + Mild decrease in clinical performance
III (severe)	Skin ++ to +++ Gastrointestinal tract and/or liver ++ to +++ Marked decrease in clinical performance
IV (very severe)	Skin ++ to ++++ Gastrointestinal tract and/or liver ++ to ++++ Extreme decrease in clinical performance

adapted from Apperley J et al.; The EBMT Handbook 2012
http://ebmtonline.forumservice.net/media/13/main.html (36)

As mentioned before, the clinical presentation of chronic GvHD can be very polymorphic. However, until the recent NIH proposal, chronic GvHD was clinically divided into limited (only localized skin involvement and/or hepatic dysfunction) and extensive disease. This classification was easy to use in clinical practice, but was insufficient to allow for appropriate comparisons between studies. (27) The current NIH global scoring system includes two components. “First each organ system (skin, mouth eyes, GI tract, liver, lungs, joints and fascia and female genital tract) receives a score from 0 to 3 precisely described according to the severity of the effected organ. Second, the number of affected sites/organs is calculated and these together establish three categories (mild, moderate and severe) according to the score generated.” (36) “Mild cGvHD reflects the involvement of no more than 1 or 2 organs/sites (except for lung) with a maximum score of 1. Moderate cGvHD involves at least 1 organ/site with a score of 2 or three or more organs/sites with a score of 1 (or lung score 1). Severe cGvHD is diagnosed when a score of 3 is given to any organ (or score of 2 to lungs).” (Table 5) (36)

Table 5: NIH consensus for global grading of chronic GvHD

Number of organs/sites	Mild	Moderate	Severe
1 site	Score 1	Score 2	Score 3
2 sites	Score 1	Score 2	Score 3
3 or more sites		Score 1	Score 3
Lung involvement		Score 1	Score 2

adapted from Apperley J et al.; The EBMT Handbook 2012
http://ebmtonline.forumservice.net/media/13/main.html (36)

1.2.5 Pathopysiology of (acute) GvHD

The pathophysiology of acute GvHD is complex and can be considered as a three step process: (1) Effects of HSCT conditioning regimen/Host APC (antigen-presenting cell) activation; (2) Donor T-cell activation; (3) Cellular and inflammatory effectors. (37) (Figure 1)

In the first step, the recipient's tissues have typically been damaged by underlying disease, the cytoreductive conditioning regimen prior to allogeneic stem cell transplantation and possibly infections. (38) This damage leads to the activation of host tissue cells and results in the release of inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin (IL)-1. As a consequence, the increased expression of MHC antigens and adhesion molecules results in the enhanced recognition of MHC and minor histocompatibility antigens by donor T-cells. The activation of donor T-cells in step 2 is then characterized by the proliferation of Th1 T-cells and the production of interleukin (IL)-2 and IFN-γ. These cytokines stimulate further T-cell expansion. In addition, natural killer cell response, the secretion of tumor necrosis factor (TNF-α) and continuing inflammation cause injury to the intestinal mucosa. Furthermore, during mucosal damage and invasion of micro-organisms, effector functions of mononuclear phagocytes are triggered via activating signals induced by bacterial LPS. This step 3 mechanism results in amplification of cytokine release and eventually leads to the "cytokine storm", characteristics of acute GvHD. (37)

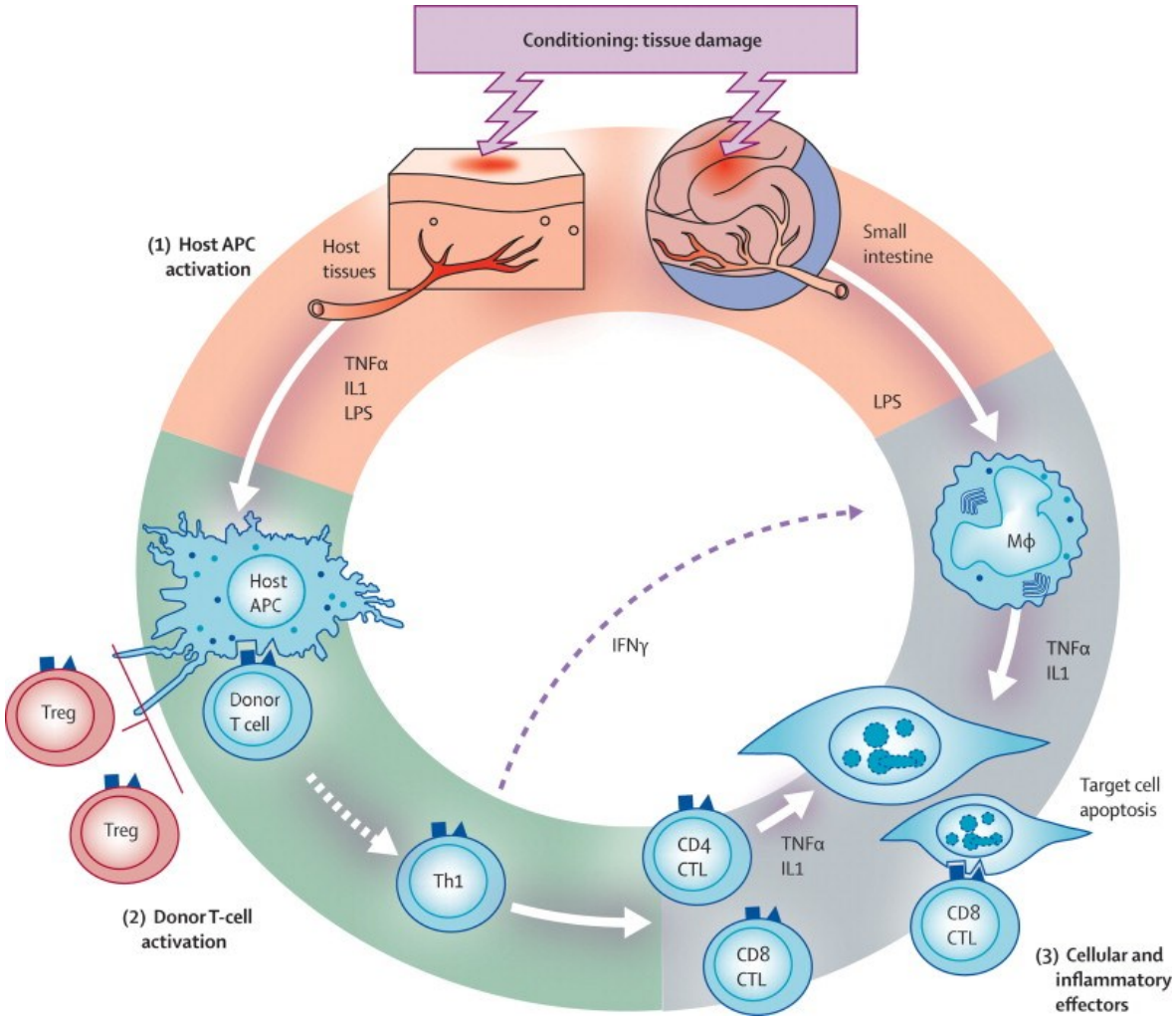

Figure 1: Pathophysiology of acute GvHD

IL 1=interleukin 1. IFN y=interferon y. LPS=lipopolysaccharide. Treg=regulatory T cell. Th1= t-helper 1 cell. CTL=cytotoxic T lymphocyte.
adapted from Ferrara JL et al., Lancet 2009 (11)

1.2.6 **Treatment of GvHD**

Glucocorticoids are the first-line treatment for acute and chronic GvHD. (39) However, despite early responses in the majority of patients, up to 60 % will have reoccurrence during tapering of glucocorticoids. (40) Mortality is still high in patients who do not respond adequately to primary therapy. (41)

1.2.6.1 Treatment of acute GVHD

With the exception of mild acute GvHD of the skin, where topical corticosteroids are used, systemic steroids (according to the guidelines, the use of methylprednisolone at a daily dose of 2 mg/kg per day is recommended) are the appropriate first-line treatment in patients with acute GvHD. (42,29) If only the upper intestinal tract is involved and symptoms are mild, the use of lower doses of glucocorticoids (methylprednisolone 1 mg/kg) together with topical agents are also sufficient. (29) If glucocorticoid therapy fails (progression of acute GvHD or no response within the first 1-2 weeks), treatment of steroid-refractory acute GvHD comprises the use of anti-TNF-α biologicals (infliximab, etanercept), ATG (antithymocyte globulin), monoclonal antibodies directed against T-cells (anti-CD 25, anti-CD 3, anti-CD 52), phototherapy (PUVA, ECP) as well as immunosuppressive drugs, such as mycophenolate mofetil (MMF), calcineurin inhibitors, pentostatin and sirolimus. (42) However, poor survival and high failure rates are characteristic for secondary treatment. (29) Therefore, novel drugs are needed and currently evaluated in clinical trials. These novel agents target diverse crucial mechanisms in the pathophysiological cascade of acute GvHD.

Magenau and Reddy divided the emerging approaches for the treatment of acute GvHD into 3 groups: (1) Extracellular mediators and receptors, (2) effectors of intracellular signal transduction and (3) regulators of transcription/translation. The first group includes the blockade of cytokines, such as interleukin (IL)-6, (IL)-21 and (IL)-23. Protease inhibition (e.g. Alpha-1-antitrypsin) is another selected target for novel therapy. Targeted intracellular signaling pathways include inhibition of Janus Kinase-2 (JAK2), STAT3 and nuclear factor NF-kB. Moreover, epigenetic therapies (DNA methylation, histone acetylation, histone methylation) have shown promise in the treatment of acute GvHD. (43) (Figure 2)

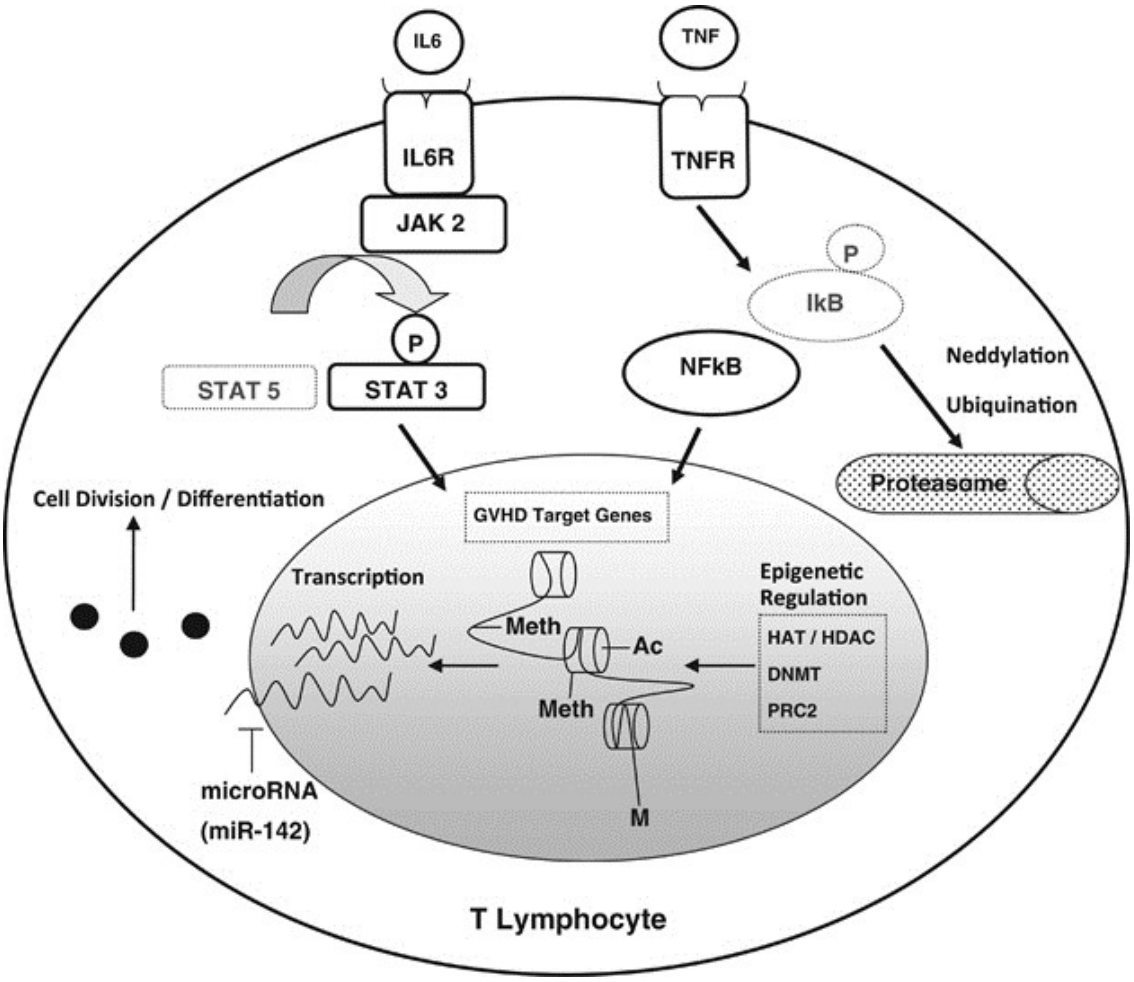

Figure 2: Molecular mechanisms involved in acute GvHD

"Intracellular signaling and transcriptional/translational regulation. JAK/STAT and NF-kB are dominant pathways recently implicated in GvHD that amplify external stimuli directing transcription of genes involved in inflammation and regulation of T-cell function. Upon translocating to the nucleus, transcription factors access target genes whose availability is controlled by the epigenetic status of the DNA and chromatin. Histone acetylation (Ac), histone methylation (M) and DNA methylation (Meth) have all been implicated in the pathogenesis of GvHD. Post-translational modification with microRNAs, neddylation and ubiquitin–proteosome pathways also tightly regulate the resulting protein content of the cell, thus further influencing its propensity to divide and differentiate into an effector of GvHD. HAT, Histone acetyltransferases, HDAC, Histone deacetylases; DNMT, DNA methyltransferase; PRC2, Polycomb Repressive Complex 2."
adapted from Magenau and Reddy; Leukemia 2014 (43)

1.2.6.2 Treatment of chronic GvHD

Topical agents are often used for treatment of patients with limited mild chronic GvHD. (44-46) However, patients suffering from systemic disease should receive methylprednisone with or without cyclosporine. (44) Systemic immunosuppressive treatment is indicated for patients who meet following criteria: Chronic GvHD that involves 3 or more organs, moderate or severe involvement in any single organ or involvement of lung. (27,47)

Primary therapy usually starts with prednisone at 0.5 to 1 mg/kg per day. A variety of complications, including gain of weight, diabetes, bone loss, hypertension, myopathy, cataract and increased risk of infection, may occur due to prolonged systemic glucocorticoid therapy. (48)

About 50 % to 60 % of patients suffering from chronic GvHD need second-line treatment within 2 years following primary systemic therapy. (49,50) ECP, rituximab, imatinib, pentostatin, mesenchymal stem cells, mycophenolate mofetil, mTOR inhibitors and interleukin-2 are agents used for second-line therapy (of chronic GvHD). The reported overall response rate is best among ECP (65-70 %) and rituximab (66-86 %) (48). This leads to a one-year survival of 70-78 % and 72 % for these treatment modalities. (48) In addition, novel developments for treatment of chronic GvHD are under active study. (48)

1.2.7 **Hyperglycemia**

Hyperglycemia is common in critically ill patients. (51) Stress hyperglycemia is usually defined as a fasting glucose above 126 mg/dl or a random blood glucose above 200 mg/dl in the presence of an acute illness, although blood glucose should be regarded as a continuous risk factor rather than a categorical. (52) Hyperglycemia in critically ill, including patients undergoing HSCT or with GvHD, may be secondary to unrecognized prediabetes, stress-induced due to the underlying disease or induced by treatment agents, which are diabetogenic such as glucocorticoids. (53)

Many studies have shown that hyperglycemia and its characteristics impact on the outcome of critically ill patients with diabetes but, even more so, in patients with stress-induced hyperglycemia. (51-53) However, little is known about the role of hyperglycemia in patients with hematological malignancies in general, as well as in patients undergoing HSCT or in patients with GvHD in particular.

1.3 ***Aim of the study***

The aim of this study was therefore to determine the impact of hyperglycemia on outcome of patients requiring systemic glucocorticoid therapy due to acute or chronic GvHD. We analyzed all relevant clinical and transplantation-related data of such patients who were treated at the Division of Hematology at the Medical University of Graz between January 2004 and January 2015. The primary objective was to identify a role of hyperglycemia in the OS of GvHD patients treated with systemic glucocorticoids.

2 Material and methods

In this study, we retrospectively evaluated 102 patients who received systemic glucocorticoid therapy due to acute or chronic GvHD after allogeneic stem cell transplantation at the Division of Hematology, Medical University of Graz between 2004 and 2015. Patient data were collected from medical records and the electronic documentation program MEDOCS (Medical Documentation and Communication System).

The following personal and medical data were retrieved:
Age, sex, diagnosis of underlying hematological disease, date of HSCT, disease remission status at HSCT, type of HSCT including stem cell source, comorbidity, date of GvHD onset, organs affected by GvHD, overall grading of GvHD, GvHD treatment, glucocorticoid dosage, blood glucose levels, body mass index (BMI) for each subject at time of HSCT as weight (kg)/(height in meters)², pre-existing diabetes, antidiabetic therapy, insulin dose, infections, overall survival, relapse-related mortality, non-relapse related mortality, causes of death, date of death, date of last contact.
For each subject, regular blood-glucose measurements during glucocorticoid therapy were recorded. For analysis, the median, mean, minimum and maximum glucose levels were taken and patients were divided into quartiles according to their glucose levels.

The main goal was to determine OS and identify risk factors for adverse outcome in this patient cohort. Both genders were equally distributed. No minimum and maximum age was considered. In addition, the incidence of other events resulting in non-relapse morbidity and mortality, such as infectious complications, were determined.

2.1 *Statistical analysis*

All patient data were entered in an anonymized manner into Microsoft Excel®2010, SPSS® Statistics 22 and GraphPad Prism Version 4. Patients alive or lost to follow-up were censored at the time they were last seen alive. The probabilities of OS and TRM were estimated using Kaplan-Meier curves. Risk factors including hyperglycemia for OS were determined by univariate and multivariate analysis using the Cox-regression model. In addition, comprehensive data on characteristics as well as treatment of hyperglycemia were

obtained and included in risk factor analysis. The study was approved by the Ethics committee of the Medical University of Graz. Due to the retrospective nature of this analysis and anonymization of data, no informed consent was needed from the included patients.

3 Results

Between January 2004 and January 2015, 306 patients underwent allogeneic HSCT at the Division of Hematology, Medical University of Graz. Of these, 102 patients (male n = 56, female n = 46) required high-dose glucocorticoid therapy due to acute or chronic GvHD and were therefore included in this study. The baseline characteristics of these subjects are summarized in Table 6.

3.1 *Patients' characteristics*

Our cohort had a median age of 47 years. The youngest patient who underwent allogeneic stem cell transplantation was 18, the oldest 72 years. Acute myeloid leukemia (AML) was the most common underlying hematological disease (n = 59, 57.84 %), followed by acute lymphocytic leukemia (ALL) (n = 18, 17.65 %). Five patients suffered from multiple myeloma (MM) and 5 from high-risk myelodysplastic syndrome (MDS). The underlying diseases of the remaining patients (n = 15, 14.71%) were Szezary syndrome, Richter syndrome, chronic myeloid leukemia, aplastic anemia, blastic plasmacytoid dendritic cell neoplasia, osteomyelofibrosis and follicular lymphoma. In our cohort only 32 recipients (31.37 %) of HSCT had a sibling donor, while in 58 cases patients received HSC from an unrelated donor. Among these, 40 donors were fully matched to the recipient, whereas for 18 subjects only a mismatched donor was available. Cord blood was used as stem cell source in 12 patients (11.77 %). The conditioning regimen was categorized as myeloablative in 46 (45.10 %) or non-myeloablative in 56 (54.90 %) patients. Thirty male patients received their transplants from a female donor. Only one patient in this series suffered from a pre-existing diabetes prior to HSCT.

Table 6: Patients‘ characteristics

Variable	Number of patients (%)
Age	
<30 years	8 (7.84 %)
30-39 years	23 (22.55 %)
40-49 years	26 (25.49 %)
≥ 50 years	45 (44.12 %)
Sex	
Male	56 (54.90 %)
Female	46 (45.10 %)
Underlying disease	
AML	59 (57.84 %)
ALL	18 (17.65 %)
MM	5 (4.90 %)
MDS	5 (4.90 %)
Others	15 (14.71%)
Donor	
Unrelated	58 (56.86 %)
Sibling	32 (31.37 %)
Cord blood	12 (11.77 %)
Conditioning regimen	
Myeloablative	46 (45.10 %)
Non-myeloablative	56 (54.90 %)
HLA- matching	
Match	72 (70.59 %)
Mismatch	30 (29.41 %)
Gender-matching	
Same gender	43 (42.16 %)
Male donor, female recipient	23 (22.55 %)

Female donor, male recipient	30 (29.41 %)
Not known	6 (5.88 %)
Remission status	
Complete remission	65 (63.73 %)
Not in complete remission	37 (36.27 %)
Pre-existing diabetes	
Diabetes	1 (0.98 %)
No Diabetes	101 (99.02 %)
BMI (Body Mass Index)	
19-25	64 (62.75 %)
25-30	28 (27.45 %)
≥30	10 (9.80 %)

3.2 *GvHD characteristics*

Acute GvHD occurred in 75 patients, while the remaining 27 subjects suffered from chronic GvHD. Among these acute cases, 28 patients were separated into grade 1-2 and the other 47 into grade 3-4 acute GvHD. The time range from transplantation to onset of GvHD was 1-34 months. The median number of involved organs or organ systems was two (range 1-3). In 50 patients two organs were affected by GvHD (49.02 %), while in the remaining 52 cases involvement of one organ was found in 34 subjects, and 18 had three or more organs involved. The majority of our patients received 2 mg per kg bodyweight methylprednisolone (n = 74, 72.55 %). Surprisingly, systemic corticoid therapy provided a response rate of 58.82 %. However, two additional immunosuppressants were needed for more than half of all patients (n = 57, 55.88 %). Cure of GvHD was defined for patients who met following criteria: No organ involvement and no treatment with any immunosuppressive drug. As a consequence of this severe disease and these strict definitions, 70 patients (68.63 %) were not cured by the last date of follow-up. GvHD characteristics are shown in Table 7.

Table 7: GvHD characteristics

Variable	Number of patients (%)
GvHD	
Acute	75 (73.53 %)
Chronic	27 (26.47 %)
Number of affected organs by GvHD	
1 organ	34 (33.33 %)
2 organs	50 (49.02 %)
3 organs	18 (17.65 %)
Overall grading (acute GVHD)	
1-2	28 (27.45 %)
3-4	47 (46.08 %)
Dosage of glucocorticoids (per kg body weight)	
≤ 1 mg/kg	28 (27.45 %)
≥ 2 mg/kg	74 (72.55 %)
Response rate of glucocorticoids	
Response	60 (58.82 %)
No response	42 (41.18 %)
Number of further immunosuppressive therapy	
1	35 (34.31 %)
2	57 (55.88 %)
3 or more organs	10 (9.81 %)
Cure of GvHD	
Cured	32 (31.37 %)
Not cured	70 (68.63 %)

3.3 *General outcome*

3.3.1 Overall survival (OS)

OS was defined as time from onset of GvHD to death from any cause. With a median follow-up of 13 months (range 1-118 months) of all patients and 43 months (range 2-118 months) of surviving patients, the probabilities of OS at 1, 2 and 5 years were 52.33 %, 40.18 % and 32.39 %, respectively. (Figure 3) Only 39 of 102 patients were alive by the last date of follow-up.

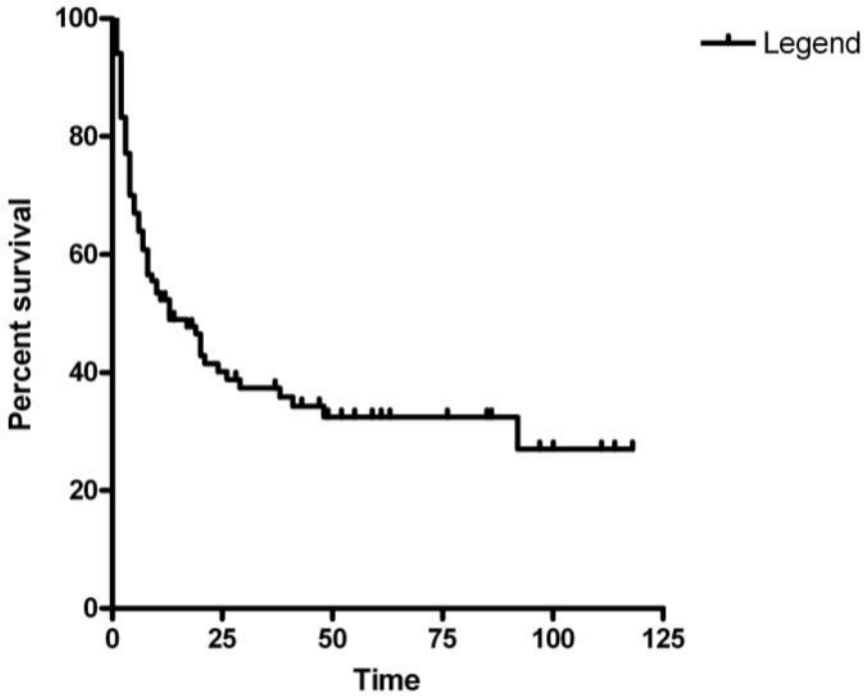

Figure 3: OS of total cohort

3.3.2 Acute versus chronic GvHD

A trend towards worse median OS, although not statistically significant, was observed in patients with acute GvHD as compared to patients with chronic GvHD (Median OS of 9 months versus 29 months, p = 0.09). The probability of 5-year OS was 31.45 % for patients suffering from acute GvHD. A summary is given in Table 8 and Figure 4.

Table 8: OS rates for patients with acute and chronic GvHD

	1-year OS	2-year OS	5-year OS
Acute GvHD	44.84 %	33.69 %	31.45 %
Chronic GvHD	69.70 %	57.40 %	36.44 %

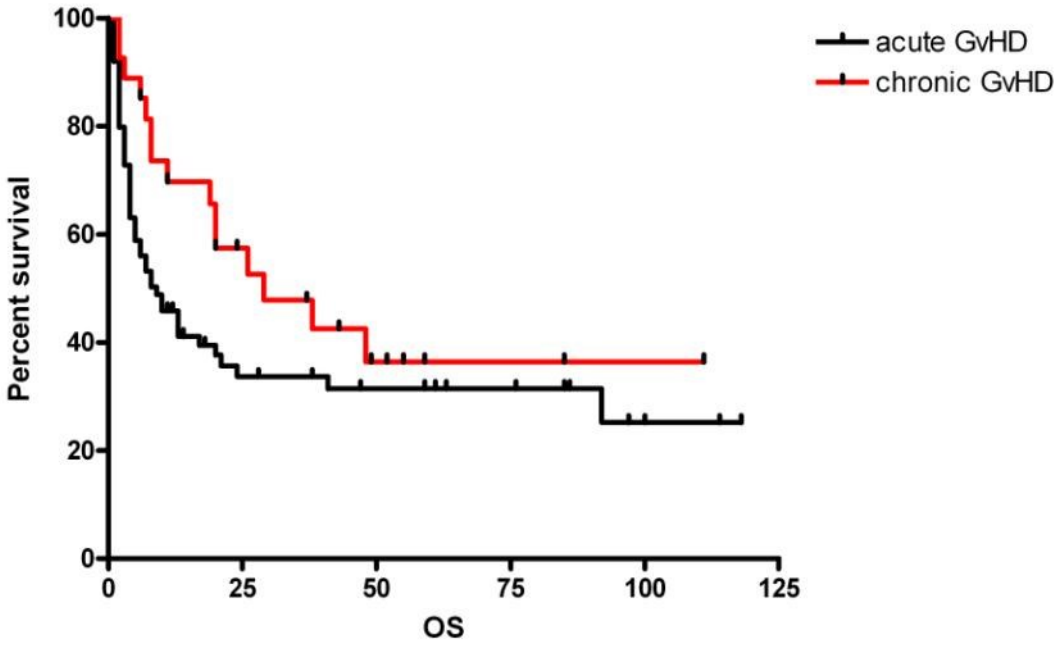

Figure 4: OS of patients with acute and chronic GvHD

3.3.3 Number of organs affected by GvHD

Next, we performed an analysis separating patients into 3 groups according to their organ involvement (1, 2 and 3 organs or organ systems). Surprisingly, the median OS was best in the group with 3 or more organs affected, although statistical significance was not reached (p = 0.3363). The probabilities of OS at 1, 2 and 5 years are listed in Table 9, Figure 5.

Table 9: OS rates for patients with GvHD and number of organ involvement

	1-year OS	2-year OS	5-year OS
One organ affected	39.83 %	36.21 %	36.21 %
Two organs affected	53.17 %	43.04 %	32.57 %
Three organs affected	72.22 %	41.27 %	24.76 %

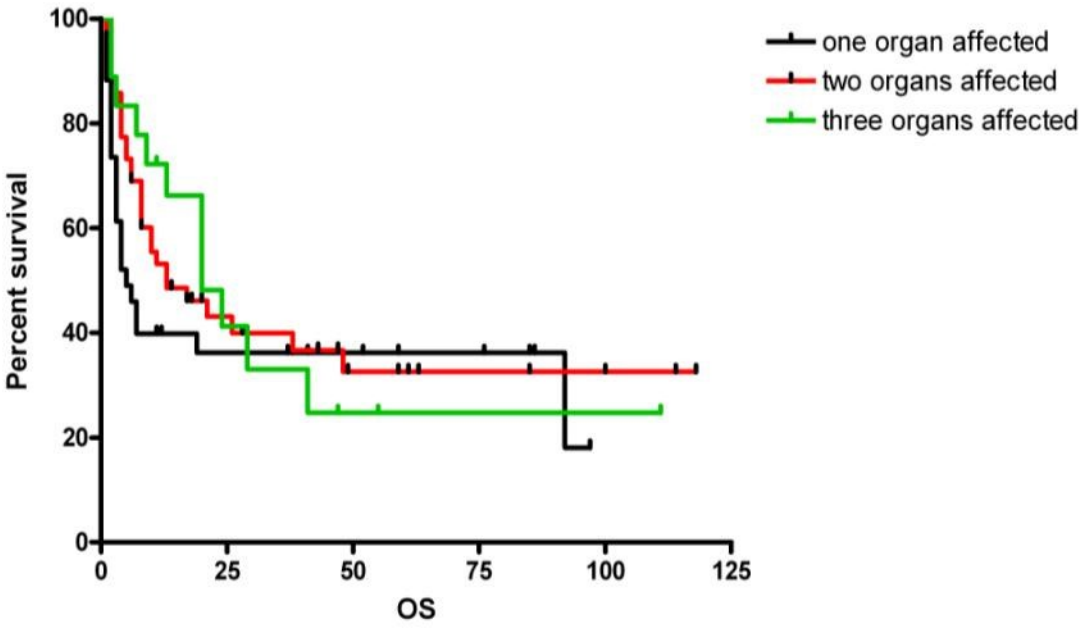

Figure 5: OS of patients with GvHD and number of organ involvement

3.3.4 Causes of deaths (relapse, non-relapse mortality), Infections

While disease relapse accounted for 9.8 % (n = 10) of the deaths within this cohort, non relapse mortality (NRM) was responsible for most cases of death (n = 53). Infection is a leading cause of death in patients with GvHD. Over the past decades, better treatment options of transplantation-related infections have improved outcomes of HSCT. Nevertheless, the occurrence of infections conferred a poor prognosis for patients with GvHD in our cohort. With a median-follow up of 13 months, the probability of 5-year OS was 26.90 % in patients with the occurrence of an infection, which was lower than in patients without an infection, although not statistically significant. (p = 0.0753) (Table 10, Figure 6)

Table 10: OS rates for patients with or without infections

	1-year OS	2-year OS	5-year OS
Infections	47.76 %	34.81 %	26.90 %
No infections	68.96 %	59.11 %	52.54 %

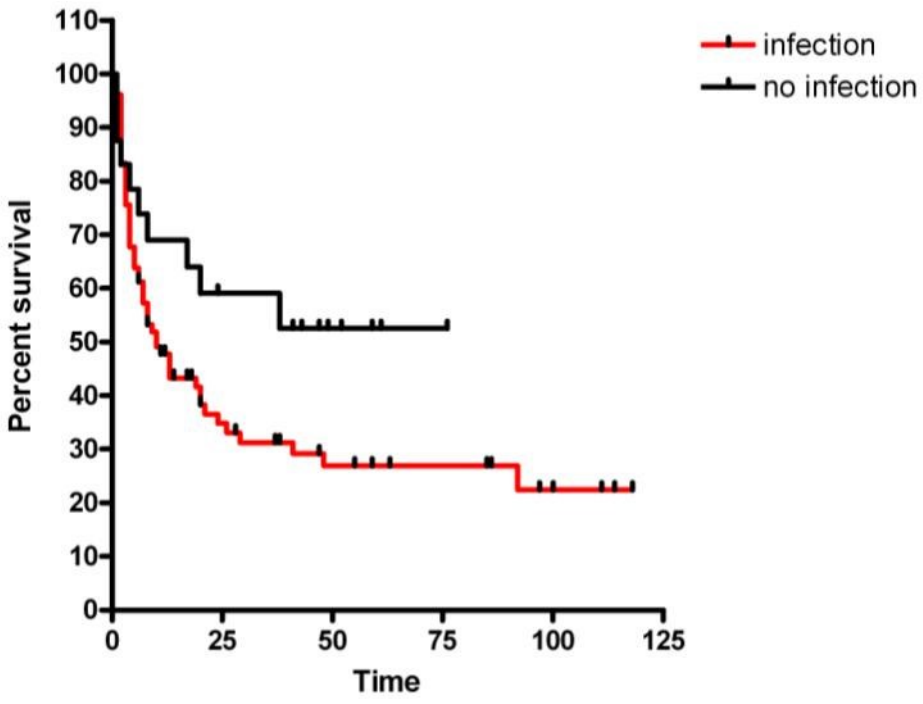

Figure 6: OS of patients with or without infections

Invasive fungal infections (IFI) adversely affect outcomes of patients with GvHD. In our cohort 19 (18.63 %) patients developed an IFI during treatment for GvHD. Median OS was significantly worse for patients with IFI (median OS of 5 months versus 24 months, p = 0.0006). (Figure 7) Hence, mortality rate was high (n = 18). Table 11 provides an overview of OS rates.

Table 11: OS rates for patients with or without IFI

	1-year OS	2-year OS	5-year OS
Invasive fungal infection	26.32 %	5.26 %	5.26 %
No invasive fungal infection	58.61 %	49.33 %	39.38 %

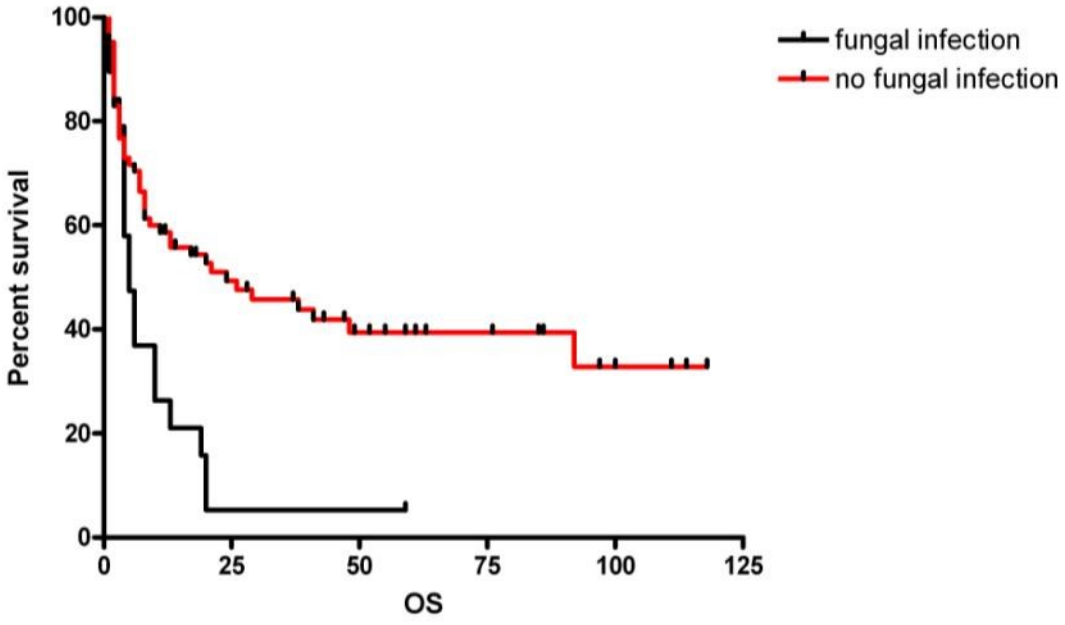

Figure 7: OS of patients with or without IFI

3.4 *Impact of hyperglycemia on outcome in patients with GvHD*

Median, mean, maximum and minimum glucose parameters as well as standard deviation were calculated for the blood glucose data available for each subject from beginning until the completion of glucocorticoid therapy. With a median of 42 blood glucose measurements (range 4-249) per patient, the median glucose levels for each parameter were as follows: median blood glucose level: 127.5 mg/dl (range 77-235 mg/dl), mean blood glucose 134.6 mg/dl (range 79-255 mg/dl), maximum blood glucose 229.5 mg/dl (range 93-793), minimum blood glucose 82 mg/dl (range 26-128 mg/dl). In total, 5524 values were obtained.

First, we evaluated the influence of median blood glucose levels on OS in this retrospective cohort. Using median glucose levels, the 102 patients were assigned into four quartiles. While median OS was not reached in the first quartile, due to low numbers of death, increasing median blood glucose levels were associated with decreasing median OS of 19, 8 and 8 months for quartiles 2, 3 and 4, respectively ($p = 0.0091$ for trend). Compared to the other three quartiles, the 5-year OS was significantly lower for patients in the fourth quartile. (Table 12, Figure 8)

Table 12: OS rates for patients with GvHD according to their median glucose level

	1-year OS	2-year OS	5-year OS
First quartile	62.48 %	56.80 %	50.48 %
Second quartile	64.10 %	39.34 %	34.97 %
Third quartile	48.25 %	38.99 %	38.99 %
Fourth quartile	34.46 %	28.72 %	7.18 %

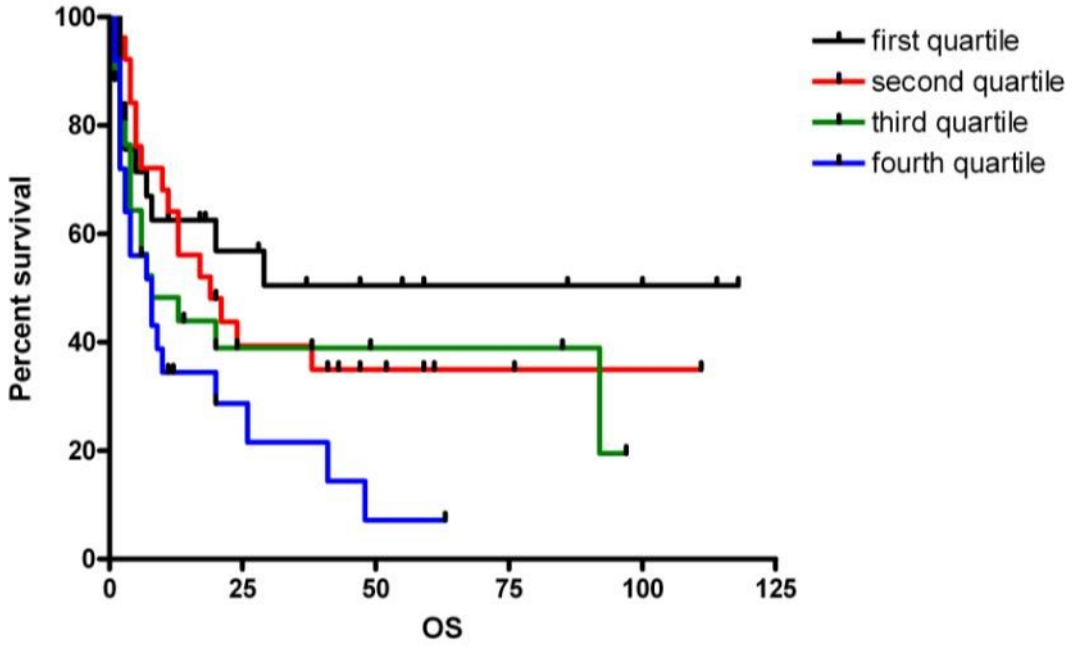

Figure 8: OS of median glucose quartiles

In addition, we performed an analysis concerning the incidence of TRM according to median glucose quartiles. High median blood glucose values were associated with increased TRM rates. In comparison to the other 3 quartiles, the incidence of TRM after 5 years was unexpectedly high for the fourth quartile (91.88 %). Median OS in quartiles 2, 3 and 4 was 38, 13 and 8 months, respectively (p = 0.002 for trend). (Table 13, Figure 9)

Table 13: Incidence of TRM according to median glucose quartiles

	Incidence of TRM after 1 year	Incidence of TRM after 2 years	Incidence of TRM after 5 years
First quartile	27.77 %	34.34 %	41.64 %
Second quartile	32.13 %	47.14 %	53.01 %
Third quartile	48.73 %	58.57 %	58.57 %
Fourth quartile	61.04 %	67.54 %	91.88 %

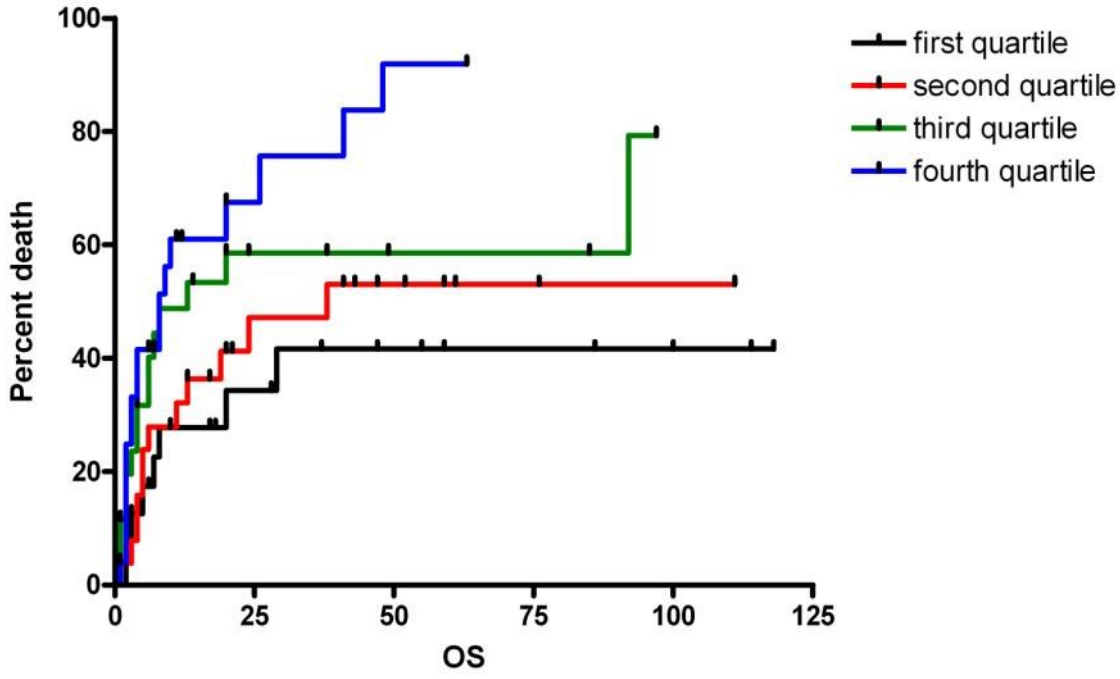

Figure 9: TRM of median glucose quartiles

Next, we analyzed the impact of mean blood glucose levels on outcome. Despite adjusted therapy, this retrospective study showed that patients with GvHD who had worse glycemic control displayed poor OS and increased risk of death. Hence, inferior survival was observed in patients corresponding to the forth quartile according to their mean glucose level. Median OS in quartiles 2, 3 and 4 was 17, 13 and 7 months, respectively. (p = 0.0012) Accordingly, the probabilities of OS at 1, 2 and 5 years were lower in patient quartiles with higher mean glucose levels, as shown in Table 14. (Figure 10)

Table 14: OS rates for patients with GvHD according to their mean glucose level

	1-year OS	2-year OS	5-year OS
First quartile	70.63 %	70.63 %	58.27 %
Second quartile	54.56 %	29.38 %	29.38 %
Third quartile	53.85%	40.50 %	32.40 %
Fourth quartile	30.33 %	24.27 %	8.09 %

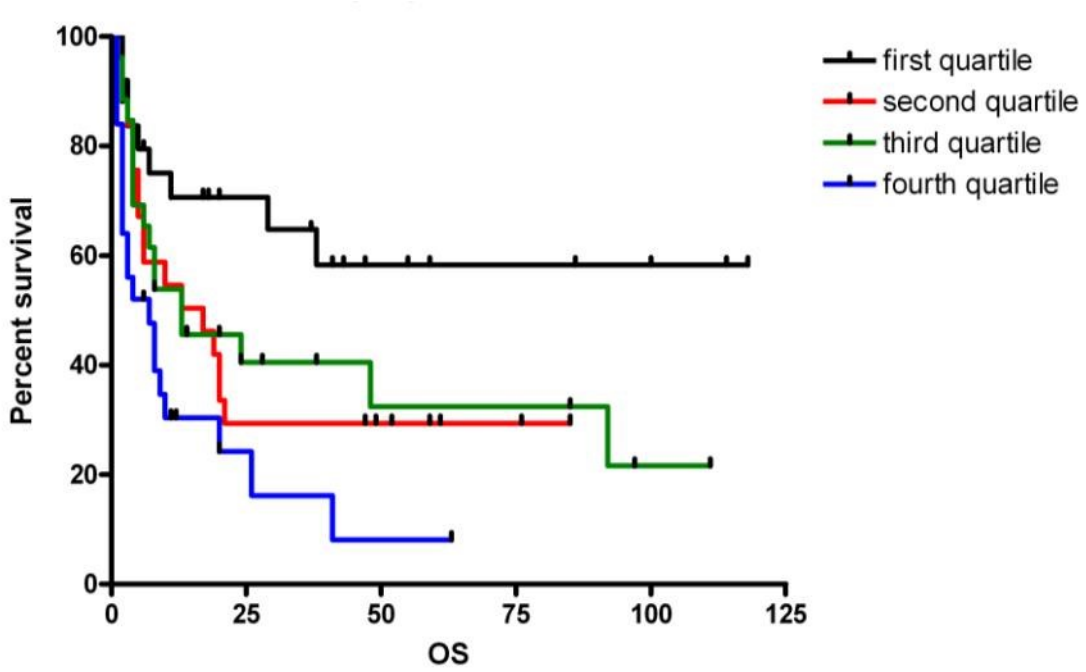

Figure 10: OS of mean glucose quartiles

Additionally, we examined the incidence of TRM according to mean glucose quartiles. While TRM was low for patients in the first quartile, increasing mean blood glucose levels were associated with increasing TRM rates for quartiles 2, 3 and 4, respectively. (Table 15, p = 0.0003). Hence, the incidence of TRM after 5 years was exorbitantly high (90.18 %) for patients of the fourth quartile.

Table 15: Incidence of TRM according to mean glucose quartiles

	Incidence of TRM after 1 year	Incidence of TRM after 2 years	Incidence of TRM after 5 years
First quartile	19.39 %	19.39 %	33.50 %
Second quartile	39.60 %	54.70 %	54.70 %
Third quartile	44.94 %	59.22 %	67.37 %
Fourth quartile	63.17 %	70.53 %	90.18 %

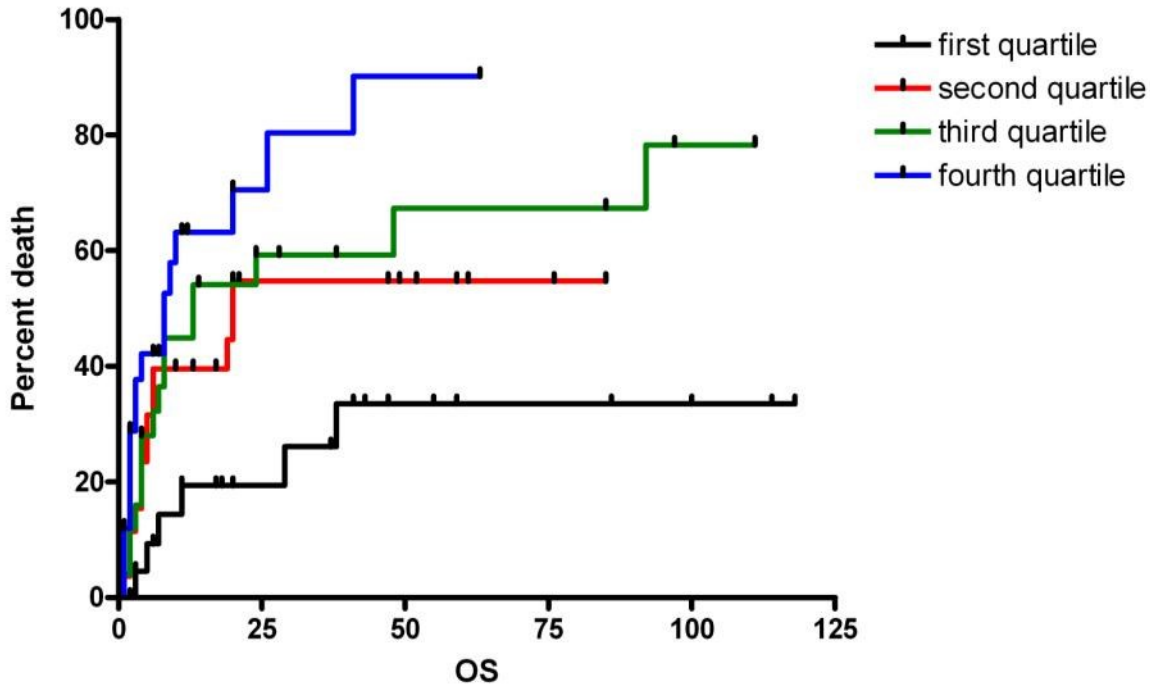

Figure 11: TRM of mean glucose quartiles

Another aim of this trial was to evaluate the impact of maximal glucose levels on outcome in patients suffering from GvHD. Median OS rates for patients with maximal glucose levels in the fourth quartile were significantly shorter as compared to those with lower maximal glucose levels (group 2) (7 versus 38 months OS, p = 0.0032). An overview of estimated OS rates is given in Table 16. (Figure 12)

Table 16: OS rates for patients with GvHD according to their maximal glucose level

	1-year OS	2-year OS	5-year OS
First quartile	69.73 %	58.10 %	51.65 %
Second quartile	60.10 %	51.15 %	46.04 %
Third quartile	46.15 %	23.08 %	15.38 %
Fourth quartile	34.67 %	28.89 %	15.41 %

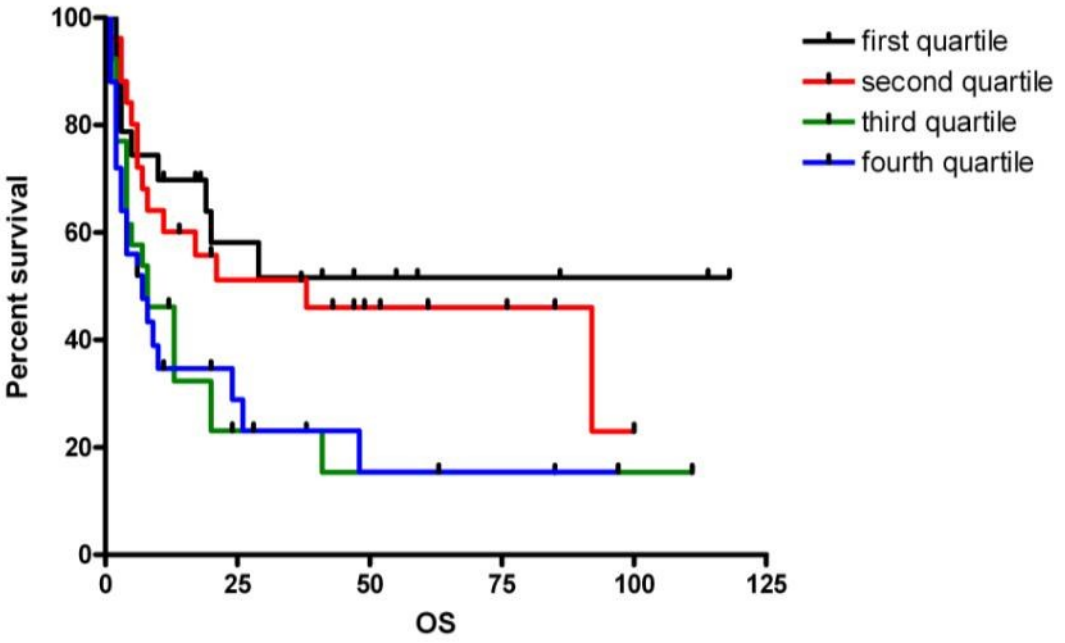

Figure 12: OS of maximal glucose quartiles

Furthermore, we analyzed TRM of maximal glucose quartiles. Median OS in the third and fourth quartile was significantly shorter when compared to the other 2 groups. (First quartile not reached, 92 months, 8 months and 9 months, p = 0.0011). Accordingly, the incidence of TRM was higher in patients with high maximal glucose levels, as shown in Table17. (Figure 13)

Table 17: Incidence of TRM according to maximal glucose quartiles

	Incidence of TRM after 1 year	Incidence of TRM after 2 years	Incidence of TRM after 5 years
First quartile	14.02 %	28.35 %	36.31 %
Second quartile	38.37 %	42.77 %	48.50 %
Third quartile	56.00 %	71.48 %	80.99 %
Fourth quartile	57.64 %	64.70 %	81.17 %

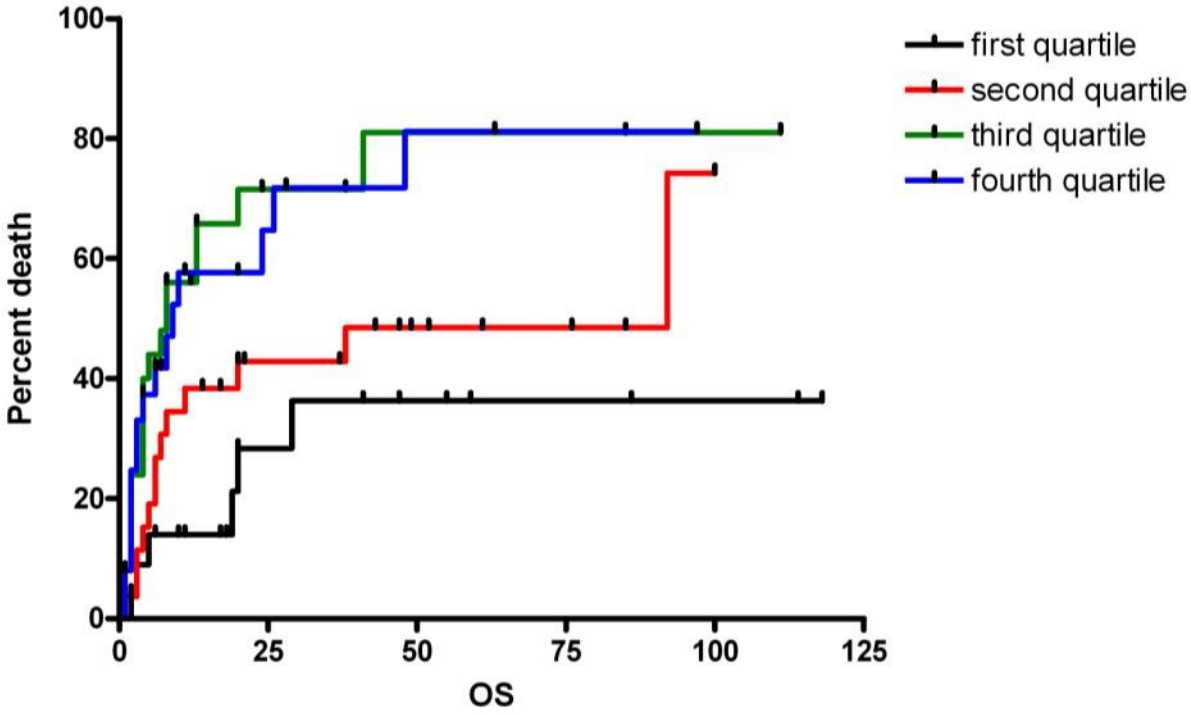

Figure 13: TRM of maximal glucose quartiles

In contrast to the other glucose parameters, minimal glucose levels had no influence on OS. Median OS for patients according to minimal glucose levels was 18, 14, 17 and 14 months, respectively. (p = 0.9309) (Table 18, Figure 14)

Table 18: OS rates for patients with GvHD according to their minimal glucose level

	1-year OS	2-year OS	5-year OS
First quartile	48.00 %	40.00 %	25.71 %
Second quartile	63.85 %	47.89 %	42.57 %
Third quartile	47.73 %	30.37 %	30.37 %
Fourth quartile	49.39%	44.45 %	33.34 %

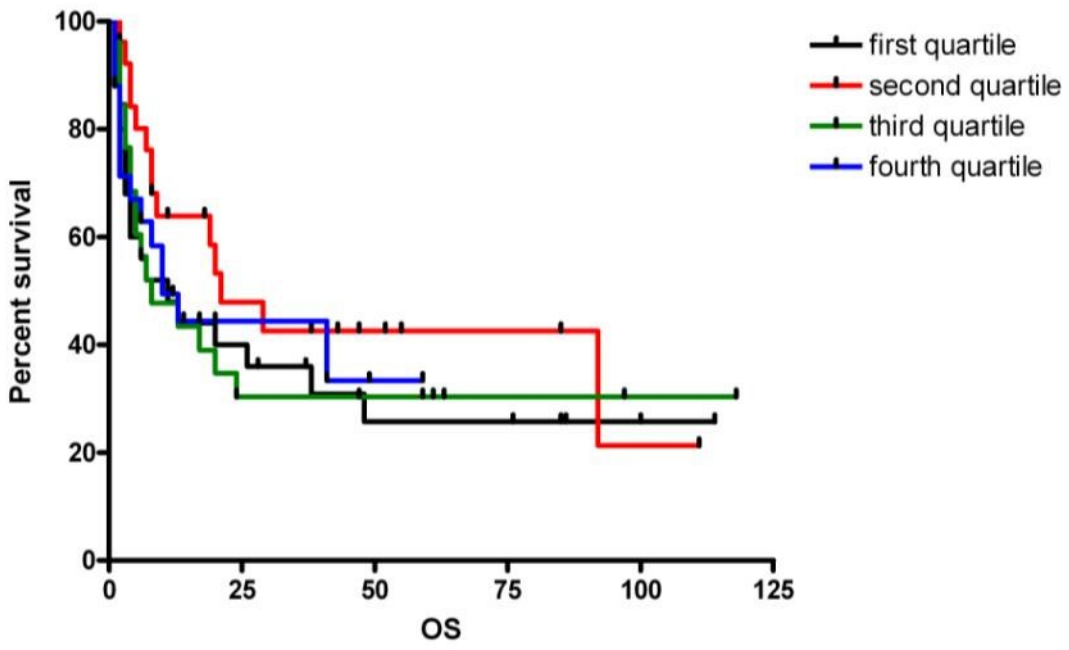

Figure 14: OS of minimal glucose quartiles

Next, we were interested in the impact of insulin treatment on the outcome of GvHD patients. Insulin, which was given in 49 patients, was associated with a shorter median OS (8 versus 38 months, p = 0.0077). Accordingly, estimated OS rates were considerably lower in patients receiving insulin, as shown in Table 19. (Figure 15)

Table 19: OS rates for patients treated with insulin

	1-year OS	2-year OS	5-year OS
Treatment with insulin	44.25 %	26.82 %	17.24 %
No insulin	60.00 %	52.81 %	47.09 %

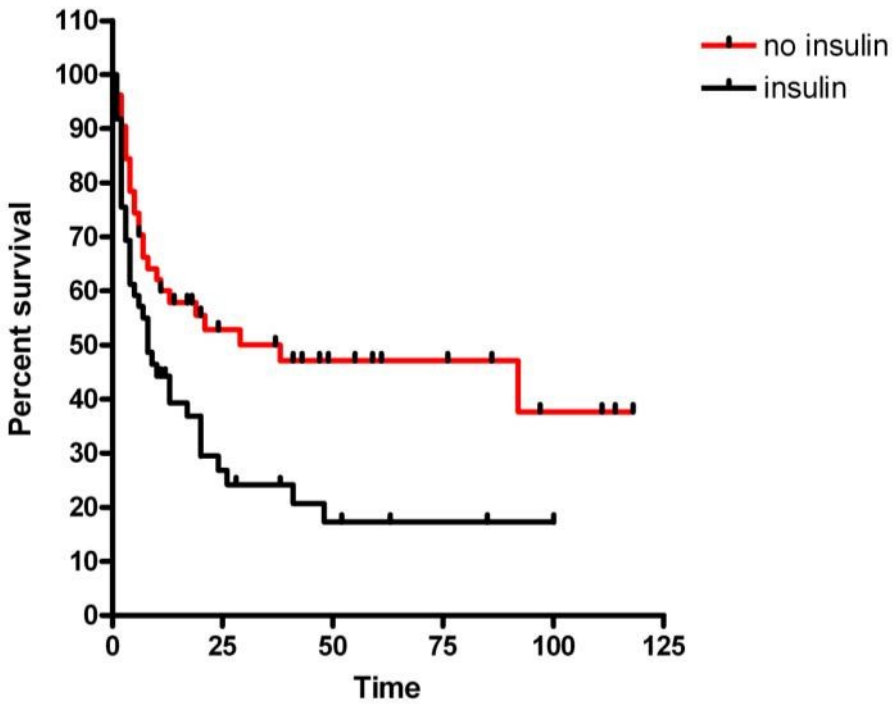

Figure 15: OS of patients treated with insulin

Furthermore, we examined the incidence of TRM of patients with insulin therapy compared to those without insulin treatment. Median OS was significantly shorter in patients receiving insulin (92 months versus 10 months, p = 0.0072, HR 0.4878, 95 % CI of ratio 0.2645 to 0.8117). An overview of TRM after 1, 2 and 5 years is detailed in Table 20. (Figure 16)

Table 20: Incidence of TRM of patients with insulin therapy

	Incidence of TRM after 1 year	Incidence of TRM after 2 years	Incidence of TRM after 5 years
Treatment with insulin	51.46 %	66.66 %	78.57 %
No insulin	33.82 %	38.84 %	45.47 %

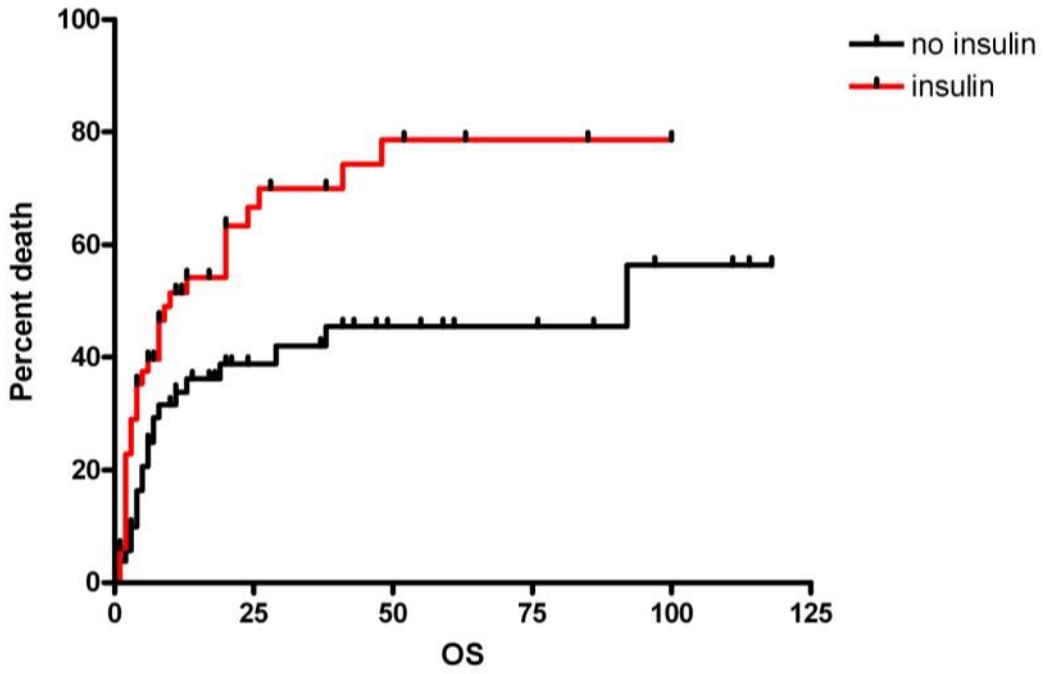

Figure 16: TRM of patients with insulin therapy

Next, we examined the influence of insulin dose on outcome within our cohort. For this purpose, patients receiving insulin were divided into two groups: 18 patients received less than 20 IU insulin per day, and 31 patients received > 20 IU insulin per day. 53 patients did not require any insulin treatment. Median OS rates for patients treated with higher insulin doses were significantly shorter as compared to those treated with lower amounts of insulin or those without insulin treatment (7 months > 20 IU insulin versus 20 months < 20 IU insulin versus 38 months without insulin treatment, p = 0.0049). A summary of estimated OS rates at 1, 2 and 5 years is detailed in Table 21. (Figure 17)

Table 21: OS rates for patients treated with insulin < 20 IU/day, > 20 IU/day and without insulin treatment

	1-year OS	2-year OS	5-year OS
No insulin treatment	60.00 %	52.81 %	47.09 %
Treatment with insulin <20 IU/day	60.61 %	30.30 %	22.73 %
Treatment with insulin >20 IU/day	35.19 %	24.63 %	14.78 %

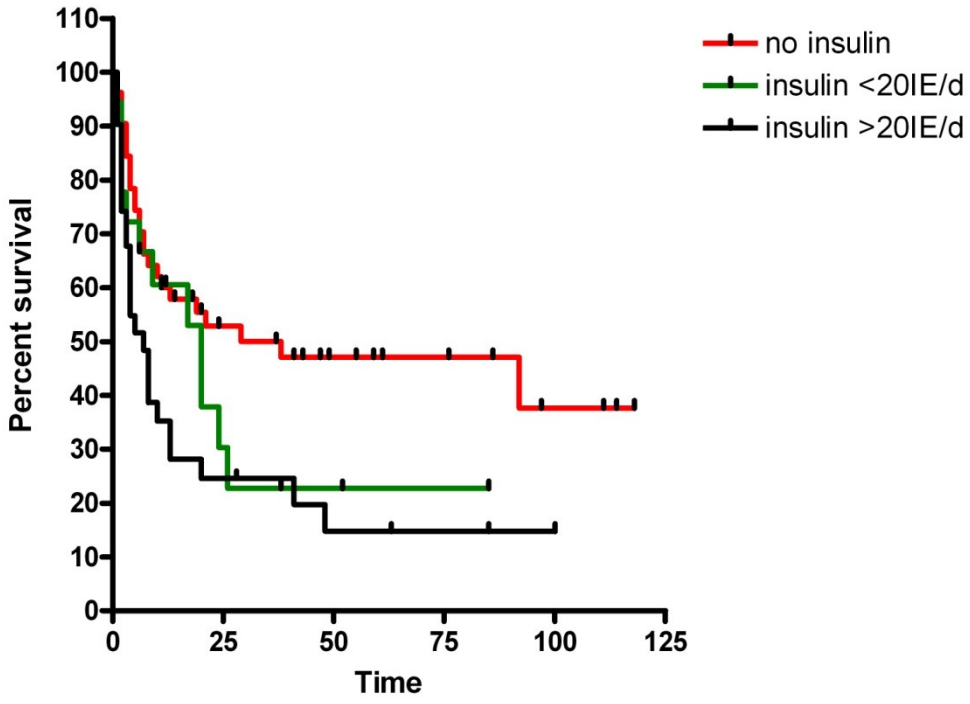

Figure 17: OS of patients treated with insulin < 20 IU/d, > 20 IU/d and without insulin treatment

3.5 *Multivariate analysis*

Finally, we performed a multivariate analysis using Cox-proportional-hazard model, including median or mean blood glucose levels as a continuous variable, age at GvHD onset, gender, donor, organs affected by GvHD, HLA-match, acute GvHD, gender-match, leukemia and BMI. The glucose variables were scaled so that the hazard ratio was interpreted as a one standard deviation change in median or mean glucose levels, respectively. Hyperglycemia defined by high median or mean blood glucose levels, donor and HLA-match were identified as independent risk factors in this multivariate analysis, as seen in Table 22 and 23.

Table 22: Multivariate analysis for patients with GvHD including median glucose levels

Covariates	**HR**	**95 % CI**		**p-value**
		Lower	Upper	
Median glucose	1.6677921	1.2714680	2.1876529	0.000220002
Leukemia	1.8506522	0.9184800	3.7288927	0.085057775
Donor cord blood	4.5397415	1.3969536	14.7529975	0.011873144
Donor sibling	0.4051491	0.1993015	0.8236056	0.012556574
Age at GvHD	0.9946431	0.9681110	1.0219023	0.697000402
Gender	0.7186665	0.3881644	1.3305740	0.293178881
Organs affected	1.0756144	0.6806846	1.6996807	0.754856558
HLA-match	0.4210155	0.1775073	0.9985733	0.049622473
Acute GvHD	1.1834251	0.6001986	2.3333859	0.626827144
Gender match	0.8282547	0.5828804	1.1769237	0.293169438
BMI	0.9844086	0.9117499	1.0628574	0.687914841

Table 23: Multivariate analysis for patients with GvHD including mean glucose levels

Covariates	HR	95 % CI		p-value
		Lower	Upper	
Mean glucose	1.7971219	1.3697478	2.3578406	0.00002329027
Leukemia	1.9638960	0.9643666	3.9993996	0.06288933
Donor cord blood	4.5754644	1.4023472	14.9284536	0.01172196
Donor sibling	0.3602029	0.1751202	0.7408973	0.00520630
Age at GvHD	0.9953702	0.9695612	1.0218661	0.7291820
Gender	0.7293769	0.3937430	1.3511115	0.3157406
Organs affected	1.0435552	0.6576133	1.6559998	0.8564029
HLA-match	0.3873007	0.1621274	0.9252094	0.03276724
Acute GvHD	1.1584169	0.5888460	2.2789143	0.6701397
Gender match	0.8169308	0.5763033	1.1580292	0.2560372
BMI	0.9815563	0.9084537	1.0605413	0.6373329

4 Discussion

Despite major efforts to establish successful treatment modalities, the outcome of GvHD is still unsatisfying. Inappropriate responses to therapy, but also an increased risk of severe infections due to intensified immunosuppressive treatment, contribute to the high morbidity and mortality of GvHD making it the most important complication in patients after allogeneic HSCT. Furthermore, since high-dose glucocorticoids are the established first-line treatment of GvHD, several other treatment-related side effects, such as steroid-induced hyperglycemia, osteopenia and increased vascular events (48), may impact the outcome of GvHD. In this diploma thesis, we have investigated the influence of hyperglycemia on the outcome of patients with GvHD requiring glucocorticoid therapy.

Our cohort comprised of 102 patients who developed GvHD after allogeneic HSCT at the Division of Hematology, Medical University of Graz and therefore received high-dose glucocorticoid therapy. Most patients were initially treated with 2 mg methylprednisolone per kg bodyweight, which is the standard first-line therapy of acute GvHD (36), while 28 patients received a lower dose mostly due to the occurrence of chronic GvHD. With a median of 42 blood glucose measurements per patient during glucocorticoid therapy, only five patients had fasting blood glucose always of less than 126 mg/dl. All others displayed at least one higher glucose level indicating the presence of steroid-induced hyperglycemia, since only one patient was diagnosed with diabetes before HSCT and GvHD onset. This finding highlights the high risk of developing hyperglycemia during glucocorticoid-based first-line treatment of GvHD.

To determine the impact of hyperglycemia on outcome in GvHD, we divided the whole cohort according to their mean, median and maximal blood glucose levels into quartiles. Assignment of patients to quartiles with higher glucose levels was associated with adverse outcome irrespectively of which blood glucose parameter was used. High levels of the mean, median or maximal blood glucose were always associated with higher mortality rates and decreased OS. For example, patients assigned to the fourth quartile according to their mean glucose level had a 3.5-fold higher risk of death when compared to patients assigned to the first quartile. In addition, patients of our cohort who needed insulin treatment had an almost two-fold risk of death. These findings relate well with the published

literature concerning both data from hematological patients either with transplantation (54-56) or without (57,58), as well as data from patients suffering from other severe diseases. (59-62)

The so far only study on the effect of dysglycemia following glucocorticoid therapy for acute GvHD was published by Pidala et al. (54) In line with our findings, they reported an adverse effect of mean and maximal glucose levels on OS in 147 patients with acute GvHD. In their cohort, patients with maximal glucose levels > 400 mg/dl had an independent hazard for non-relapse death more than > 9 times the reference group with glucose levels between 100-200 mg/dl. Furthermore, in good agreement to our data, the need for insulin therapy led to a three-fold increase of risk of death in their cohort. Since insulin treatment was significantly related to the glucose parameters, treatment with insulin was strongly dependent on the degree of hyperglycemia. Therefore, the insulin effect observed likely reflects the associated severity of hyperglycemia. Interestingly, in contrast to our results, they also found a non-linear correlation of minimal glucose levels, wherein there was an increased hazard for death in both the group of patients with minimal glucose levels between 0-60 mg/dl, as well as patients with levels between 81-150 mg/dl as compared to the reference group with levels between 61-80 mg/dl. One explanation for this discrepancy could be that in our cohort only six patients had glucose levels < 60 mg/dl, probably reflecting a less stringent therapeutic glucose control algorithm applied at our institution. Therapy-induced hypoglycemia has been identified as a major risk factor for outcome in critically ill patients undergoing tight glycemic control. (53,63,64)

In a very recent report of registry data on more than 7 600 patients Takano et al. showed that pre-existing diabetes was associated with an increased risk of death after allogeneic stem cell transplantation. (65) Patients with diabetes had a worse 1-year OS after HSCT as compared to patients without diabetes (44.7 % versus 63.9 %, $p < 0.01$). In multivariate analysis, higher numbers of death were due to increased NRM in patients with pre-existing diabetes. Interestingly, while infection-related NRM was higher in the diabetes group, there was neither an increased incidence of acute GvHD nor an elevated number of deaths due to acute GvHD in patients with pre-existing diabetes. Since only one patient in our cohort had diabetes prior to HSCT, we cannot draw any conclusions from our study on the impact of pre-existing diabetes on the outcome in GvHD.

In general, patients with diabetes have an increased susceptibility to various types of infections. The skin, the lungs and the urinary tract are the major affected organ systems. A summary of studies investigating susceptibility of diabetic subjects to acquire infections is detailed in Table 24. In addition, diabetes may affect the outcome of patients with specific infections. Trials showing an adverse association between diabetes and outcome are listed in Table 25. However, although diabetes prior to HSCT was associated with an increased risk of fungal infections in the study of Takano et al. (65), there were no significant differences in the cumulative incidences of bacterial and viral infections at one year after HSCT between the groups. We also found a relatively high number of IFI in our cohort (19 out of 102 patients), but we could not clearly establish an association of high blood glucose levels with the incidence of IFI or infections in general. Nevertheless, the occurrence of an IFI was associated with an adverse outcome in our cohort.

Table 24: Studies investigating susceptibility of diabetic subjects to acquire infections

Author	Year	Infection type	n	Study design	Main outcome measures	Main findings
Zhao et al. (66)	2009	Skin infection	8 655	Longitudinal matched control	Incidence of skin infections	Higher risk for skin infections (adjusted OR 2.8)
Kornum (67)	2008	CAP	34 329	Population-based matched control	Pneumonia-related hospitalization	Increased risk for CAP-related hospitalization (RR 1.26 [95 % CI 1.21-1.31])
Benfield (68)	2007	Infectious diseases	10 063	Prospective	Hospitalization, 28-day mortality	Higher risk for infection-related hospitalizations and UTI-related mortality (HR 3.9 [95 % CI 1.2-12.7]); no difference in mortality because of sepsis, CAP, skin infection and other infections
Boyko (69)	2005	UTI	1 017	Longitudinal matched control	Incidence of UTI	Higher risk of UTI (RR 1.8 [95 % CI 1.2-2.7]) and antibiotic treatment (RR 2.3 [95 % CI 1.3-3.9])
Thomson (70)	2004	Pneumococcal bacteremia	598	Matched control	Bacteremia	Higher risk for pneumococcal pneumonia (OR 1.9 [95 % CI 1.4-2.6])
Shah (71)	2003	Infectious diseases	513 749	Matched control	Hospitalization, mortality	Higher risk for hospitalization (RR 2.17 [95 % CI 2.10-2.23]) and infection -related mortality (1.92 [1.79-2.05]); no difference in in-hospital mortality (1.05 [0.89-1.01] and 0.84 [0.87-1.01])

adapted from Schuetz P et al.; Diabetes Care 2011 (72)

Table 25: Studies showing an adverse association between diabetes and outcome of infections

Author	Year	Infection type	n	Study design	Main outcome measures	Main findings
Kornum (73)	2007	CAP	29 900	Population-based cohort	Complications, bacteremia, mortality	Higher mortality rates (1.2 [95 % CI 1.1-1.3]), but similiar rates of complications and bacteremia; mortality within patients with diabetes increased when initial glucose levels > 14 mmol/L in multivariate analysis (adjusted MMR 1.46 [95 % CI 1.01-2.12] compared with patients <6.1 mmol)
Thomson (74)	2005	Enterobacteria bacteremia	1 317	National registry	Bacteremia, 30-day mortality	Higher risk for bacteremia (OR 2.9 [95% CI 2.4-3.4]) and a trend toward higher 30-day mortality (1.4 [1.0-2.0])
Fine (75)	1996	CAP	33 148	Meta-analysis	30-day mortality	Higher risk for mortality (OR 1.3 [95 % CI 1.1-1.5])

adapted from Schuetz P et al.; Diabetes Care 2011 (72)

Interestingly, there is evidence that hyperglycemia in critically ill patients with pre-existing diabetes might not be as detrimental as hyperglycemia in patients without a history of diabetes. Table 26 gives an overview of studies demonstrating an increased mortality rate when comparing patients who were hyperglycemic during their acute illness without diabetes to those with diabetes. However, we are not aware of any study demonstrating that this phenomenon is also true for patients after HSCT or patients with GvHD. Since our cohort had only one patient with pre-existing diabetes, our data are insufficient to draw any conclusion in this respect.

Table 26: Studies that have evaluated the relationship between hyperglycemia and mortality when categorizing patients according to pre-existing diabetes

Study author	Intensive care unit/s	Patients known to have diabetes, n (%)	Patients not known to have diabetes, n (%)	Hyperglycemia consistently associated with reduced survival *	Hyperglycemia appears more harmful in patients without diabetes **	Magnitude of harm ***
Rady et al. (59)	1	1 083/2 826 (38.3)	1 743/2 826 (61.7)	No	Yes	5-fold
Krinsley (60)	1	578/2 699 (22.2)	2 128/2 699 (78.8)	Yes	Yes	2-fold
Egi et al. (61)	2	728/4 946 (14.7)	4 218/4 946 (85.3)	No	Yes	3-4-fold
Falciglia et al. (62)	173	78 142/259 940 (30.0)	180 898/259 940 (70.0)	Yes	Yes	2-fold

* Increasing blood glucose concentrations were associated with reduced survival in patients not known to have diabetes, as well as those known to have diabetes. ** For all patients with hyperglycemia, patients without known diabetes had reduced survival when compared to those known to have diabetes. *** Magnitude of the increased mortality rate when comparing patients who were hyperglycemic during their acute illness without diabetes to those with diabetes.

adapted from Deane AM & Horowitz M; Diabetes, Obesity and Metabolism 2013 (51)

Given the fact that we and others have identified hyperglycemia as adverse risk factor in patients with GvHD or – more general – after allogeneic HSCT, it is tempting to speculate that tight glycemic control (TGC) in such patients might improve outcome. However, we are not aware of any studies which tested TGC in patients with GvHD or after allogeneic HSCT. Despite recommendations of TGC for critically ill patients by several experts and some medical societies, benefits of TGC have not been proven to be definitive. Supporting data remain conflicting, since critical reviews on the published literature indicate that patients suffering from severe diseases can have variable responses to TGC and that a survival benefit in one population cannot be extrapolated to another. (53,63) For example, a large study by Finfer et al. (NICE-SUGAR Study Investigators) reported an increased mortality (27.5 %) in adult ICU patients who received IGC (81-108 mg/dl) when compared with the control group (24.9 % mortality). Furthermore, severe hypoglycemia (values < 40 mg/dl) was more frequent in patients with IGC (6.85 %) than in the control group (0.5 %). (76) Thus, glucose management goals need to be carefully evaluated in prospective clinical

trials for specific patient populations including patients with GvHD and after HSCT to clearly identify benefits of TGC.

In our study, the increased mortality of hyperglycemic patients was due to higher rates of TRM, because the number of deaths related to relapse was low and was not associated with hyperglycemia in our cohort. These findings correlate perfectly with the published literature. Neither hyperglycemic GvHD patients included in the study of Takano et al., nor hyperglycemic patients after allogeneic HSCT in general as reported by Hammer et al. had an increased risk of relapse of their underlying disease as compared to normoglycemic patients. (56,65) Together, these data strongly indicate that hyperglycemia does not alter the biological behavior of the underlying hematological malignancy after allogeneic HSCT, but does affect transplant-related complications.

Although our findings on the impact of hyperglycemia on the outcome of patients with GvHD compare well with the published literature and add novel important aspects to this clinical issue, our trial has several limitations due to its retrospective nature. First of all, a consistent protocol for blood glucose measurements, blood glucose control and nutritional support was not applied to all GvHD patients. Secondly, data on the date and clinical parameters of infections were not sufficiently available for all patients. Therefore, we had to limit reporting on infections, which occurred during the first year after start of glucocorticoid-based GvHD therapy. This could be the reason that we were not able to clearly establish an association of hyperglycemia with the occurrence of infections in our cohort as has been reported in other studies (56,65), although we could show an adverse impact of the occurrence of infections in general or invasive fungal infection in particular on OS of GvHD patients. Thirdly, in general the sample size of our study cohort with 102 patients was rather small. These patient numbers did not allow elucidating subtle effects of dysglycemia on survival parameters in GvHD patients. For example, we only had six patients with documented hypoglycemia (blood glucose at least once < 60 mg/dl) in our cohort. This small number may be the reason why we were not able to detect an effect of hypoglycemia on outcome in GvHD as has been reported by others (54). Even though there were several limitations, the quality of collected data was good, and we think that our findings are important considering the remaining challenging issue of decreasing mortality and morbidity in patients with GvHD. We propose that prospective studies testing the impact

of hyperglycemia as well as a strict blood glucose management in GvHD are crucial and will help to improve the outcome in this clinical setting.

4.1 *Conclusion*

In this retrospective analysis, we observed an adverse impact of glucocorticoid-induced hyperglycemia on the outcome of patients with GvHD. Using multivariate analysis, hyperglycemia was identified as the major adverse risk factor for TRM in GvHD patients, while the number of relapse-related deaths was not affected. The results from this diploma thesis may therefore lay the foundation for prospective studies determining the impact of hyperglycemia and tight glucose control in GvHD patients undergoing systemic glucocorticoid therapy and may affect clinical practice in the future.

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