

Doctoral thesis

Effect of tight glyceemic control in in- and outpatient settings in different patient populations

A retrospective analysis to evaluate the impact of hyperglycemia in patients with acute myeloid leukemia and Graft-versus-Host disease

and

Results from a prospective randomized pilot trial focusing on the management of hyperglycemia in patients with Graft-versus-Host disease

submitted by

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STATUTORY DECLARATION

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz“.

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LIST OF ABBREVIATIONS

| | |
|----------|---|
| ACTH | Adrenocorticotrope hormone |
| ADA | American Diabetes Association |
| AE | Adverse event |
| AI | Adrenal insufficiency |
| AML | Acute myeloid leukemia |
| ATGL | Adipose triglyzeride lipase |
| BCD | Beta cell dysfunction |
| BIT | Bolus insulin therapy |
| BMD | Bone mass density |
| BMI | Body Mass Index |
| BW | Body weight |
| CDA | Canadian Diabetes Association |
| CI | Confidence interval |
| CMV | Cytomegalie virus |
| CR | Complete remission |
| CRH | Corticotropin-Releasing-hormone |
| CS | Corticosteroid |
| DM | Diabetes mellitus |
| DM2 | Diabetes mellitus type 2 |
| DPP4 | Dipeptidyl-peptidase-4 |
| DPP4-i | Dipeptidyl-peptidase4- inhibitor |
| EBV | Epstein-Barr Virus |
| ECOG | Eastern cooperative oncology group |
| EGP | Endogenous glucose production |
| GC | Glucocorticoid |
| GCP | Good clinical practice |
| GI | Gastrointestinal |
| GIP | Glucose-dependent insulintropic polypeptide |
| GIT | Gastrointestinal tract |
| GLP1 | Glucagon-like-peptide-1 |
| GLP1-RA | Glucagon-like peptide 1 receptor agonist |
| GvHD | Graft versus host disease |
| HbA1c | Glycated hemoglobin 1c |
| HLA | Human leukocyte antigen |
| HPA-axis | Hypothalamic-pituitary-adrenal-axis |
| HSC | Hormone sensitive lipase |
| HSCT | Hematopoietic stem cell transplantation |
| HSL | Hormone sensitivity lipase |
| IL | Interleukin |
| IQR | Interquartile range |
| IU | Insulin units |
| kg | Kilogramm |
| LDH | Lactat dehydrogenase |
| MC | Mineralocorticoid |

| | |
|---------------|--|
| MUG | Medical University Graz |
| NEFA | Non-esterified-fatty-acids |
| NRM | Non relapse mortality |
| NSAIDs | Nonsteroidal anti-inflammatory drugs |
| OAD | Oral antidiabetic agent |
| OGTT | Oral glucose tolerance test |
| ON | Osteonecrosis |
| OR | Odds ratio |
| OS | Overall survival |
| PIT | Prandial insulin therapy |
| PPAR γ | Peroxisome proliferator-activated receptor- γ |
| PSC | Posterior subcapsular cataract |
| RA | Rheumatoid arthritis |
| RNA | Ribonucleic acid |
| SAT | Subcutaneous adipose tissue |
| SGLT2 | Sodium-like glucose transporter 2 |
| SIHG | Steroid induced hyperglycemia |
| SMBG | Self monitored blood glucose |
| DNA | Deoxyribonucleic acid |
| SU | Sulfonylurea |
| SZT | Stammzelltransplantation |
| TIA | Transitoric ischemic attack |
| TZD | Thiazolidinedione |
| USA | United States of America |
| WHO | World health organization |

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ABSTRACT GERMAN

Hintergrund:

Erhöhte Blutzuckerwerte stellen bei hospitalisierten PatientInnen mit und ohne diabetischer Erkrankung ein erhöhtes Risiko für gesundheitliche Komplikationen und Mortalität dar. Die diesbezügliche Datenlage bei PatientInnen mit hämatologischen Erkrankungen ist dürftig.

Die akute myeloische Leukämie (AML) ist eine Erkrankung des blutbildenden Knochenmarks bei welcher es zu einer unkontrollierten Bildung unreifer Zellen kommt. Dieser Umstand führt unbehandelt zu potentiell lebensbedrohlichen und meist letalen Blutungs- und Infektionskomplikationen.

Eine etablierte kurative Therapie der AML besteht aus der Transplantation von allogenen Stammzellen (SZT). In etwa 30-40% der PatientInnen tritt nach SZT eine sogenannte akute Graft-versus-Host Reaktion (GvHD) auf, welcher eine immunotoxische Reaktion des Spendergewebes auf den Organismus des Empfängers zugrunde liegt. Dadurch kommt es zur Entstehung inflammatorischer Prozesse und nekrotischer Schädigung unterschiedlicher Organsysteme (meist Gastrointestinaltrakt, Haut, Leber).

Die Erstlinien-Therapie der GvHD beruht auf der Gabe hochdosierter systemischer Steroidtherapien, welche bei circa der Hälfte der Fälle die Entwicklung erhöhter Blutzuckerwerte zur Folge hat.

Der Einfluss der Hyperglykämie auf das Outcome von AML- und GvHD- PatientInnen nach SZT wurde bisher nur unzureichend erforscht. Zudem ist es bis heute nicht klar ob eine engmaschige Kontrolle und intensiviertere Therapie erhöhter Blutzuckerwerte einen Einfluss auf das erhöhte Komplikations- und Mortalitätsrisiko hat.

Material und Methoden:

In zwei retrospektiven Datenanalysen wurde einerseits bei PatientInnen mit AML und andererseits mit GvHD der Einfluss der Hyperglykämie auf unterschiedliche Endpunktparameter überprüft.

In die AML Studie wurden 159 PatientInnen eingeschlossen, bei denen aufgrund einer AML eine SZT durchgeführt wurde. Innerhalb der ersten 28 Tage nach SZT wurden die Blutzuckerwerte dokumentiert. Entsprechend der mittleren Glukosewerte während dieser Periode wurden die PatientInnen in 3 verschiedene Glucosekategorien aufgeteilt und auf das Outcome entsprechend der glykämischen Situation untersucht.

Die GvHD Studie hat 104 PatientInnen untersucht, bei denen aufgrund einer akuten GvHD eine hochdosierte Steroidtherapie eingeleitet wurde.

In dieser Studie wurde das Outcome der Studienteilnehmer je nach Blutzuckerhöhe während des stationären Aufenthaltes untersucht. Weiters wurde ein Score entwickelt, der einerseits die mittleren Glukosewerte der ersten 3 Tage nach Einleitung der Cortisontherapie erfasst und andererseits das Ansprechen auf die Steroidtherapie innerhalb einer Woche beinhaltet.

Um zu untersuchen, ob eine Verbesserung der Blutzuckerkontrolle das Outcome der PatientInnen verbessert, ist die Durchführung einer großen, multizentrischen Interventionsstudie notwendig. Als ersten Schritt dafür wurde eine prospektive randomisierte Pilotstudie durchgeführt, in welche hyperglykämische PatientInnen mit akuter GvHD zu einer intensiven Glukosekontrolle unter dem Einsatz eines Entscheidungsunterstützungssystems zur Insulintherapie (GlucoTab®) oder zu konventionellem Glukosemanagement randomisiert wurden.

Ergebnisse:

In der AML Studie konnte gezeigt werden, dass Patienten mit einem einzigen erhöhten Blutzuckerwert innerhalb der 28 Tage ein 2.5-fach erhöhtes Mortalitätsrisiko aufwiesen und das Risiko stieg mit der Höhe des mittleren Blutzuckers. Nach Adjustierung für verschiedene Begleitumstände (Alter, HLA Kompatibilität, Stammzellherkunft, ...) blieb die Hyperglykämie ein starker Prädiktor für Mortalität.

In der GvHD Studie konnte gezeigt werden, dass GvHD PatientInnen mit Hyperglykämien ein 2.5-fach erhöhtes Risiko für Mortalität zeigten. Unser implementierter Score (3-Tages Blutzuckerwerte und Ansprechen auf Steroide) war ebenfalls höchst prädiktiv für ein schlechtes Outcome. So zeigte sich, dass PatientInnen ohne Erfüllung dieser Kriterien (0 Punkte) ein 5 Jahres- Überleben von 75% hatten, während von den PatientInnen mit einem Score von 2 nach 5 Jahren lediglich 4% noch am Leben waren.

Die prospektive Studie demonstrierte eine effektive und sichere Anwendbarkeit des GlucoTab Systems zur Behandlung steroidinduzierter Hyperglykämien bei PatientInnen mit akuter GvHD.

Zusammenfassung:

Die beiden retrospektiven Studien haben gezeigt, dass die Hyperglykämie ein potenter und unabhängiger Prädiktor für ein ungünstiges Outcome darstellt. Ob die Hyperglykämie

möglicherweise ein modifizierbarer Risikofaktor ist, in prospektiven multizentrischen Studien untersucht werden. Möglicherweise kann die Verwendung des GlucoTab Systems zur Verbesserung der Blutzuckerkontrolle in dieser Population beitragen.

ABSTRACT ENGLISH

Background:

Hyperglycemia represents a risk factor for complications and mortality in hospitalized patients.

Data on hyperglycemia contributing to outcome in patients with hematological diseases is sparse and needs more attention.

Acute myeloid leukemia (AML) is a malignant disease of the hematopoietic system leading to uncontrolled formation of immature myeloid cells. This condition leads untreated to potentially life-threatening bleeding and infectious complications.

The most established curative therapeutic option for AML includes allogeneic hematopoietic stem cell transplantation (HSCT). About one third of patients undergoing HSCT develop a so called graft-versus-host disease (GvHD) which is a potentially life-threatening condition. In GvHD, an immunotoxic reaction of donor cells to recipient's tissue leads to immune reactions, severe inflammatory processes and tissue necrosis, mainly affecting the gastrointestinal tract, the skin and the liver. As first-line therapy for GvHD includes high-dose systemic steroid therapy, about half of patients develop steroid-induced hyperglycemia. However, the impact of hyperglycemia on outcome of patients with AML and GvHD has not been sufficiently investigated so far. Furthermore it still remains unclear whether a tight glycemetic control of hyperglycemia might contribute to a better prognosis in patients with AML and GvHD.

Material and Methods:

We performed two retrospective trials including patients with AML and separately GvHD, aiming to investigate the influence of hyperglycemia on different outcome endpoints.

In the AML-study 159 patients with AML undergoing HSCT were included. Within the first 28 days after HSCT glucose measurement were documented. All-cause mortality was analyzed according to the extent of hyperglycemia after HSCT.

The second retrospective trial focused on 104 patients who received high dose steroid therapy for GvHD. During time of steroid therapy glucose values were collected and the risk for mortality and other outcome parameters of interest associated with hyperglycemia were calculated. Furthermore, in this cohort a score comprising glycemetic status during the first 3 days after steroid initiation and steroid treatment response within 7 days, as a predictor for overall survival, was developed.

Additionally, we performed a prospective randomized trial including 10 patients with GvHD to determine whether an automated decision support system for insulin therapy (GlucoTab®) can be established effectively and safely for the treatment of steroid induced hyperglycemia in patients with GvHD.

Results:

In the AML-study we were able to demonstrate that hyperglycemia during the first month after HSCT is one of the major predictors for mortality. Patients showing one single hyperglycemic value during 28 days of observation had a 2.5-fold elevated risk for mortality. Results remained significant after adjusting for several co- factors (age, stem cell source, HLA- compatibility...) in a multivariate analysis.

In the GvHD-study, patients with hyperglycemia during the first 3 days after initiation of steroid therapy had a 2.5- fold higher risk for mortality. Additionally, our implemented score (based on 3-day hyperglycemia and lack of response to steroids) showed to be highly predictive for mortality, indicating that patients meeting none of the criteria (0 points) had a 5-year survival of 75%, while patients with a score of 2 were alive in only 4% after 5 years of follow-up.

The prospective pilot study demonstrated that GlucoTab could be established safely and effectively for the treatment of steroid-induced hyperglycemia in patients with GvHD.

Conclusion:

Both retrospective analyses identified hyperglycemia to be a prominent and independent risk factor for overall mortality. Whether hyperglycemia is just a predictor for adverse outcome or might be a modifiable risk factor has to be evaluated in larger multicenter intervention studies. Perhaps, management of hyperglycemia using GlucoTab might contribute to an improvement of glycemia in this indicated population.

1.INTRODUCTION

Impact of hyperglycemia in hospitalized patients

Hyperglycemia in hospitalized patients is estimated to be present with a prevalence of 13-25%(1). For every two patients with diabetes in the hospital, there is supposed to be one other patient with unrecognized hyperglycemia(2-4).

Hospitalized patients presenting with hyperglycemia in general are exposed to a higher risk of morbidity and mortality.

Multiple epidemiological studies investigating glucose as predictor for adverse outcome have been performed in various patient populations and different medical settings including internal and surgical departments(5-10).

In critically ill patients a retrospective analysis of more than 1.800 patients, showed a strong association between increases in average glucose concentration and mortality(5, 11).

Interestingly, patients with newly detected hyperglycemia diagnosed during hospitalization face an even worse outcome as compared to those with manifest diabetes, published by Umpierrez et al.. They show longer length of hospital stay, higher rates of intensive care unit (ICU) admissions and higher needs for translational or nursing home after hospital stay compared to patients who were known diabetic or non-diabetic. In this analysis also inpatient mortality rate was more than 4 times higher (16%) in hyperglycemic patients without previously known diabetes (2).

However, in a meta-analysis including 15 studies, hyperglycemia was associated with a 3.9-fold increased in-hospital mortality in patients with diabetes and 1.7-fold in hyperglycemic patients without preexisting diabetes. In addition, the risk for congestive heart failure and cardiogenic shock was highly associated to in-hospital hyperglycemia(7).

In a retrospective study which included more than 1.500 patients undergoing coronary artery bypass grafting, every 50 mg/dl increase of mean blood glucose was related to a 37% increase in mortality and 23% higher risk of infection rate and a 0.76 days prolonged hospital stay(9).

In a study investigating 656 stroke patients, 30-day mortality was 87% higher in patients who were hyperglycemic and also their length of hospital stay and risk of being admitted to ICU was significantly higher(10).

In general, little is known about the impact of intensive antihyperglycemic treatment on outcome in hospitalized patients. A prospective randomized analysis of 1.548 critically ill patients on an ICU of a surgical ward demonstrated a reduction of in hospital mortality from 8 to 4.6% in hyperglycemic patients who were intensively treated with insulin. Furthermore this study showed a statistically significant decreased risk for overall mortality, bloodstream infections, acute renal failure, requirement of blood transfusions and critical-illness polyneuropathy in patients who were randomized to the intensive treatment arm(12). However, this study was criticized for being a single center study and focusing on surgical patients only. The NICE-SUGAR study, a multicenter trial in surgical and medical patients, did not confirm the results but highlighted the importance of avoiding hypoglycemia(13).

Patients suffering from hematological malignancy are especially exposed to a high risk for hyperglycemia due to factors as concomitant immunosuppressant therapy (especially corticosteroid therapy), total parenteral nutrition and medical stress as infection(14).

Especially hematological patients requiring steroid therapy, frequently develop hyperglycemia due to miscellaneous effects of steroids affecting metabolism. Previous studies estimated the prevalence of hyperglycemia in hematological patients ranging from 12 to 64%, depending on underlying disease and therapeutic approach(15-18).

The impact of hyperglycemia in patients with hematological malignancy has been investigated only in a small number of retrospective analyses.

One study conducted in hospitalized patients with acute myeloid leukemia (AML) showed, that hyperglycemia was associated with an increased risk of in-hospital mortality and sepsis(19). Hammer et. al. demonstrated that malglycemia, defined as hypo- and hyperglycemia contributed to a higher non-relapse mortality in patients who underwent hematopoietic stem cell transplantation (HSCT)(20). Hyperglycemia immediately appearing after HSCT also turned out to be highly predictive for acute stem cell graft rejection reactions(21).

STUDIES PERFORMED IN THE SCOPE OF THIS DOCTORAL THESIS

Aim of this thesis was to investigate the impact of hyperglycemia in patients with hematological diseases. On the one hand we retrospectively investigated the impact of hyperglycemia on outcome in patients who underwent hematopoietic stem cell transplantation for acute myeloid leukemia and in patients who developed Graft-versus-host-disease, on the other hand we performed a prospective pilot trial in order to test the performance of a decision support system for insulin therapy (GlucoTab) in patients with acute Graft-versus-Host disease and steroid-induced hyperglycemia.

2. EARLY HYPERGLYCEMIA AFTER INITIATION OF GLUCOCORTICOID THERAPY PREDICTS ADVERSE OUTCOME IN PATIENTS WITH ACUTE GRAFT-VERSUS-HOST DISEASE(22)

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Early Hyperglycemia after Initiation of Glucocorticoid Therapy Predicts Adverse Outcome in Patients with Acute Graft-versus-Host Disease.

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2.1. Glucocorticoids

2.1.1. Glucocorticoid metabolism

Glucocorticoids (GC) are a member of steroid hormones that are produced in the zona fasciculata of the cortex of the adrenal glands regulated by the hypothalamic-pituitary-adrenal axis. Signal hormones for adrenal GC production (cortisol) include corticotropin releasing hormone (CRH), which is provided by the hypothalamus and adrenocorticotrophic hormone (ACTH) which is secreted by the anterior pituitary gland. The secretion of ACTH is pulsatile with an interval of 30-120 minutes and follows a diurnal rhythm with higher ACTH levels in the morning than during night. The hypothalamic-pituitary-adrenal axis is based on a negative feedback regulation system, meaning that CRH, ACTH and cortisol reciprocally influence the amount of production of these particular hormones. In adrenal deficiency (e.g. Addison's disease), cortisol synthesis is pathologically reduced, while CRH and ACTH production is compensatory high. Contrary, if there is an excess of GC synthesis as it is present in patients with endogenous hypercortisolism (Cushing's-Disease) or exogenous GC therapy ACTH production is reduced to a minimum.

The basis for GC synthesis is cholesterol, which is on the one hand provided by nutrition and on the other hand produced from acetyl- CoA in the liver. Cortisol is transformed during intramitochondrial processes to his metabolically active form by undergoing several intermediate prehormonal stages (pregnenolone, progesterone, 17- hydroxyprogesterone and 11- desoxycortisol)(23).

2.1.2. Physiologic action of glucocorticoids

Physiologically, GCs are the most efficient stress hormones which are evolutionary responsible for “fight and flight” situations. This life saving strategy is initiated by providing substrate for oxidative metabolism by stimulating hepatic production of glucose, increase of lipolysis of adipose tissue, employment of proteolysis and modulation of blood pressure. Any stress, exposed to the organism leads to an increase of plasma GC levels, which essentially causes tolerance of and resistance to stress situations. GC effects are widespread and GC homeostasis is essential for living(24). The following chapter describes the main physiologic effects of endogenous glucocorticoids (EGC). Imbalances of glucocorticoid household and its consequences as well as therapeutic GC approach are mentioned in a later chapter.

2.1.3. Effect of endogenous glucocorticoids on the carbohydrate metabolism

GCs induce hepatic gluconeogenesis by stimulating the conversion of protein to carbohydrates and promote the glucose storage for the supply of essential tissues such as brain and red blood cells at the expense of comparatively less essential organs such as muscle tissue. This effect is especially important for times of higher stress exposure or starvation. The effect on hepatic gluconeogenesis is leading to elevation of plasma glucose which stimulates the secretion of liver glycogen(25). This effect can be explained by a direct effect of GCs on the hepatic expression of genes, which are coding for enzymes for glucose and glycogen biosynthesis.(26) The diabetogenic effect of GC will be explained in an upcoming chapter of this thesis.

2.1.4. Effect of GC on lipid metabolism

It is well known that patients with endogenous or exogenous glucose excess present with redistribution of fat especially with central adiposity(27). This effect is not well understood. However, GCs elevate the concentration of fatty acids in the organism by promoting lipoprotein lipase activity which consecutively leads to availability to distribute ectopic fat (muscle, liver, central adipose tissue). By increased expression of fatty acid synthase also the production of lipids in the hepatocytes is stimulated. Furthermore GCs induce the transformation of pre- to mature adipocytes also leading to hyperplasia of adipose tissue(28).

2.1.5. Effect of GCs on the cardiovascular system

Corticosteroids (CS), including glucocorticoids (cortisol) and mineralocorticoids (MC)(aldosterone), regulate and influence plasma volume, electrolyte retention, and epinephrine production, which is responsible for the maintenance of normal blood pressure, balancing of electrolytes and sufficient cardiac ejection. CS' have an impact on myocardial function, vessel tone and capillary permeability. Further they have an influence on prostaglandin synthesis decreasing vasodilation and increasing of responsiveness to vasopressors(29, 30). This theory can be confirmed by the exemplary fact that patients with hypercorticism tend to be hypertensive and patients with corticosteroid deficiency (e.g. patients suffering from Addison's disease) mostly present with hypotension and hyponatremic irregularity (29, 30).

2.1.6. Anti- inflammatory effects of GC

By regulation of lysosomal membranes and consecutive release of proteolytic enzymes, GCs inhibit inflammation and allergic reactions(31, 32). By decreasing capillary permeability, movement of leukocyte trafficking, synthesis of prostaglandins and leukotrienes may be selectively inhibited reducing tissue edema. GCs also promote the apoptosis of several inflammatory cells which may further contribute to a reduction of inflammatory cell burden. Furthermore GCs also elevate the release of secretory products of granulocytes, macrophages and mast cells which leads to an increased liberation of inflammatory mediators like histamine and serotonin(32-34).

2.1.7. Anti-immunity and immunosuppressant effects of GC

GCs show pleiotropic effects on the immune system. Steroids interfere with the synthesis of cytokines which play an important role in the proliferation and interaction of T-cells and additionally suppress B-cells by downregulating antibody production. Furthermore, GCs decrease activity of granulocytes by inhibiting chemotaxis, adhesion and release of toxic substances. These properties make exogenous steroids to an essential regulatory and counter regulatory therapeutic option for several medical problems, as well as in primary and secondary prophylaxis of immune-mediated and inflammatory diseases(35, 36).

2.2. Pharmaceutic mechanism, types and indications for corticosteroid therapy

2.2.1. Pharmaceutic quality of CS

2.2.1.1. Types of CS therapy

A multiplicity of different GC derivatives, mimicking endogenous cortisol, are available on the market. The agents differ with potency, duration of effect and ratio of MC to GC properties, which specify each drug from each other. The probably most widely used systemic GC is prednisone. Prednisone has a high GC portion and a low MC effect. It is generally used as an immunosuppressant and anti-inflammatory agent, however in patients which are prone to fluid retention, related agents as prednisolone or methylprednisolone are preferably prescribed because of their minor properties of MC(37). Dexamethasone similarly has only a little percentage of MCs and is overall much more potent concerning its GC activity and has a more extended effect as prednisone and prednisolone; because of this higher potency it has a higher suppressive effect on adrenal activity which makes it to a therapeutic option of choice only in severe or acute situations. Furthermore, it is not recommended for continuous use by it's hard to regulate activity which is a result from its extended effect.

As corticoid replacement therapy, especially in patients with adrenal insufficiency (e.g. Addison's disease) mainly cortisone and hydrocortisone are used because of their accompanying MC component. Hydrocortisone and cortisone is much less potent than prednisolone or prednisone and resembles the endogenous cortisol the most. This circumstance allows a preferable therapeutic regulation and measurability in patients who continuously require physiologic amounts of CS therapy. Fludrocortisone is the agent with the highest MC proportion, that's why it is also often used in patients suffering from general adrenocortical hormone deficiency(38, 39).

| | Approximate equivalent dose (mg) | Relative GC activity | Relative MC activity | Duration of action (hours) | General therapeutic indications |
|----------------------------|--|----------------------------|----------------------------|----------------------------------|--|
| Glucocorticoids | | | | | |
| <i>Short-acting</i> | | | | | |
| Hydrocortisone | 20 | 1 | 1 | 8-12 | <ul style="list-style-type: none"> • Relatively high mineralocorticoid activity makes it suitable for use in adrenal insufficiency • Similar to hydrocortisone |
| Cortisone | 25 | 0.8 | 0.8 | 8-12 | |
| <i>Intermediate-acting</i> | | | | | |
| Prednisone | 5 | 4 | 0.8 | 12-36 | <ul style="list-style-type: none"> • High glucocorticoid activity makes it useful for long-term treatment, and as an anti-inflammatory/immunosuppressant |
| Prednisolone | 5 | 4 | 0.8 | 12-36 | <ul style="list-style-type: none"> • Similar to prednisone |
| Methylprednisolone | 4 | 5 | Minimal | 12-36 | <ul style="list-style-type: none"> • Anti-inflammatory/immunosuppressant |
| <i>Long-acting</i> | | | | | |
| Dexamethasone | 0.75 | 30 | Minimal | 36-72 | <ul style="list-style-type: none"> • Anti-inflammatory/immunosuppressant; used especially when water retention is undesirable • Usually reserved for short-term use in severe, acute conditions given its high potency and long-duration of action • Similar to dexamethasone |
| Betamethasone | 0.6 | 30 | Negligible | 36-72 | |
| Mineralocorticoids | | | | | |
| Fludrocortisone | 0 | 10-15 | 125-150 | 12-36 | <ul style="list-style-type: none"> • Used for aldosterone replacement |

Table 1 Dosing equivalents, properties GC/MC and general therapeutic indications of available GCs according to (22)

2.2.1.2 Indications for GC therapy

CS' have a broad spectrum of efficacy in acute and chronic illness and both in therapeutic as well as in prophylactic indications. In this thesis I will not focus on indications of steroid therapy in detail.

2.3. Adverse effects of GC therapy or endogenous GC excess

Overall adverse side effect profile can be summarized as CUSHINGOID as indicated in table 2. Manifestations of steroid-related side effects vary dependent on the type of administered steroid, duration of use, patient age and dose. In a study, performed by Saag et al., 224 patients randomly received whether low-dose and long-term prednisolone compared to placebo in the treatment for rheumatoid arthritis (RA). Ninety-two adverse events (AE) were documented in the intervention group while only thirty-one AEs occurred in the control group. Main AEs were fractures, serious infections and gastrointestinal harm(40). In this chapter main adverse effects of exogenous or endogenous and its consequences are mentioned. For easier understanding adverse effects can be described following the abbreviation "CUSHINGOID"(35).

| Letter of mnemonic | Sign of symptom | Pathophysiology |
|--------------------|---------------------------------------|---|
| C | Cataracts | Unknown but may involve perturbed migration of lens epithelial cells |
| U | Ulcers | Disputed, but may be due to the inhibition of gastric-protective prostaglandins, mucus production and/or bicarbonate secretion |
| S | Striae and skin thinning | Unclear but may involve decreased fibroblast proliferation and/or altered metabolism of the extracellular matrix |
| H | Hypertension and hirsutism (in women) | Increased plasma volume, cardiac output and peripheral vascular resistance occur through both mineralocorticoid and glucocorticoid effects: hirsutism occurs due to dysregulated production of adrenal testosterone |
| I | Immunosuppression and infections | Discussed in the main text |
| N | Necrosis of femoral heads | increased bone marrow fat, decreased bone perfusion and osteocyte apoptosis |
| G | Glucose elevation | Glucose intolerance and insulin insensitivity |
| O | Osteoporosis and obesity | Inhibition of osteoblast function and survival, decreased bone mass (osteoporosis) and redistribution of adipose tissue (obesity) |
| I | Impaired wound healing | Reduced proliferation of fibroblasts and epidermal cells, inhibition of collagen synthesis and reduced angiogenesis |
| D | Depression and mood changes | Psychological, cognitive and behavioural disturbances |

Table 2 Adverse effect profile of GC therapy according to (35)

2.3.1. Eye complications – Cataract and Glaucoma

The main ocular complication of a prolonged GC therapy is the development of cataract, mainly the posterior subcapsular cataract (PSC) which is characterized by a round, discoid, opaque mass which is built of malformed and deformed lens fibers situated in the central posterior layer of the lens. The etiology of steroid induced cataracts is not fully understood. Possible ocular damage associated with GC therapy leading to PSC may be explained by alterations to the intraocular levels of growth factors or by induction of pathologic genes in lens epithelial cells(41). Treatment options for PSC other than surgical removal of the lens are not available yet(42). Prevalence of PSC in patients using GCs vary from 16 to 38% depending on underlying disease, patient age, gender (women>men), type of steroid therapy, dose and duration of use(43-45).

Another less frequent steroid associated eye complication is appearance of glaucoma which is a result from increase of the intraocular pressure(46).

2.3.2. Gastrointestinal bleeding

The upper gastrointestinal (GI) bleeding and perforation complications associated with the use of oral steroids was reported to develop with an odds ratio (OR) of 1.8 in a

retrospective analysis of 2.105 cases (compared with 11.500 age- and sex matched controls, splitted up in a ratio of 2.4 for gastric and 1.2 for duodenal damage). OR for bleeding and perforations was 1.8 and 1.6. This risk is dramatically increased when patients followed a concomitant therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) (OR 4.0 and 12.7 in patients with low and high- dose of NSAID intake)(47). The mechanism how GCs induce GI complications has not been fully elucidated. However, GCs adversely affect tissue repair leading to impairment and delay of wound healing(48, 49). Another theory indicates that the anti-inflammatory steroid effect and its analgesic properties may mask symptoms of GI ulcers which may contribute to a delay of diagnosis(50). Altogether there seem to be several opinions on the steroid therapy potential to damage GI tissue which indicate different considerations of concomitant GI ulcer prophylaxis recommendations in recent guidelines in patients on steroid therapy(51).

2.3.3. Skin complications

GC therapy causes atrophic alterations of the skin which leads to cutaneous thinning and fragility, insufficient healing mechanisms, acne, purpura and striae. While topical use of steroids cause only seldom and negligible systemic damage, the skin itself is potentially bereaved by local use of GC therapy(52). GC induced skin damage is based on inhibition of proliferative epidermis metabolism as well as suppression of collagen and fibroblast synthesis(53).

2.3.4. Hypertension and cardiovascular risk

By its fluid retentive effect, CS' (especially MC) lead to hypertension especially in patients with a previous history of hypertensive predisposition and accompanying metabolic syndrome(54, 55). This circumstance consequently increases risk for cardiovascular disease (CVD). A population based case-control study which included more than 50.000 patients with CVD in medical history and on GC therapy, showed a significant association (adjusted OR 2.66) of GC therapy and heart failure compared to matched controls without concomitant GC therapy. The impact of GC therapy on fatal ischemic coronary artery disease was less but still significant (OR 1.2). In this analysis risk for stroke or transient ischemic attack (TIA) was not adversely associated(56). That CVD risk is highly depending on dose of GC therapy (>7.5 mg/day of prednisolone or equivalent) was shown

by Wei et al. in 2004(57), also current vs. previous use of CS' was shown to be an impacting factor(56).

GC therapy also seems to influence risk of arrhythmias, especially the probability to develop atrial fibrillation and atrial flutter(58, 59). As it is often in clinical research and patient studies we cannot clearly define whether an applied therapy or the underlying conditions (comorbidities) can be blamed for the adverse outcome even if studies were matched on several patient characteristics, therefore expressiveness of this data is always limited.

2.3.5. Hirsutism

Hirsutism, mainly diagnosed in women, is associated either to increased production or elevated sensitivity of the hair follicles to circulating androgens(60), leading to excessive hair growth where hair is usually absent or minimal, for example in the face, the chin or the chest. Degree and severity of hirsutism can be quantified by the ferriman-gallwey-score(61). Causes mainly include overproduction of adrenal or ovarian steroid hormones, or steroidal progenitor hormones (dihydroepiandrosterone (DHEAS), 17-A-hydroxyprogesterone (17-OHP), testosterone, e.g. in patients with polycystic ovary syndrome, as well as in relation to exogenous exposure to GC therapy(62, 63).

2.3.6. Acne

Acne is commonly associated with GC excess, either endogenously or exogenously, mostly by GC related arising of hyperandrogenemia. This acne presents as small follicular papules, mainly on the forehead, cheeks and chest and as comedones(64).

2.3.7. Immunosuppression

As mentioned in chapter 2.1.7., GCs regulate immunologic mechanism by inhibiting immune system cells; this fact leads to a beneficial decrease of inflammation but concurrently predisposes for infection. A meta-analysis which included more than 70 clinical trials with more than 2.000 participants which were randomized either to receive GC therapy or not, revealed a significant higher risk for infectious disease (OR 1.66) in patients who were treated with GC agents. Also in this case the dosing of steroids (>10

mg/day prednisolone or equivalent) and the duration of GC administration played a crucial role(65). Also patient age, the underlying disease, comorbidities and co-medication was identified as significant contributors of adverse outcome in patients using GC therapy(40, 66). Especially patients with bone marrow disorders requiring stem cell transplantation face a higher risk to develop fungal and viral infections(67).

2.3.8. GC related myopathy

The direct catabolic effect of GCs on muscle tissue can cause decrease of muscle protein synthesis and inhibition of protein catabolism. This makes GCs to be the most potential causer for drug- induced myopathy(68). Initial characteristics of GC induced myopathy, which can present acute or chronic, include atrophy and weakness in muscular tissue mainly in muscles of the upper and lower extremities, while myalgia and sensation were not outlined(69, 70). As it is instant in most of the GC side effects, intensity and incidence of myopathy correlates directly with amount and duration of GC therapy, however dosages of >10 mg/day of prednisolone or equivalent were proven to be decisive(71).

2.3.9. Cognitive and psychiatric side effects of GC therapy

GCs have a substantial effect on multiple psychiatric and neurocognitive alterations. Starting with slight disorders as mood swings, insomnia, irritability and aggressiveness, GC therapy can cause severe psychiatric impairments as delirium, suicidal ideation and intense bipolar problems. These adverse effects can develop within short time and often need only low doses of GC therapy to occur, however neurological symptoms are definitively dose dependent(72, 73).

An association of declarative memory loss is described, which usually occurs during the first weeks of GC therapy(74), occurring also in low dose but more in high dose GC therapy (prednisolone 5-40 mg/day)(75). Naturally, older patients are more prone to appropriate memory disorders. The so called “steroid dementia syndrome” was prescribed by Varney et al. and was defined by a GC associated deficit in memory retention, attention, concentration and mental speed(76). Well-known and serious, but biologically not well understood, are acute psychotic derailments which mainly occur in hospitalized patients who received high doses of GC (>20 mg/day Prednisolone or equivalent). Luckily GC

associated mental alterations and disorders are usually a reversible matter and stop after habituation to therapy, dose reduction or cessation(77).

2.3.10. GC induced suppression of adrenal function

GC therapy leads to physiologic suppression of adrenocortical gland activity and superior centers of the HPA axis. GC therapy induced adrenal insufficiency (AI), also called tertiary or iatrogenic AI is the most frequent cause of AI. Small doses of GCs are enough to determine measurable suppression of the HPA axis. As a result, especially in prolonged use of GCs, the adrenal cortex loses ability to produce cortisol long-termly(78). This situation is often unrecognized and becomes then problematic, when GC therapy is stopped abruptly or patients with AI are exposed to higher steroid requirements as it is the case in intermittent severe illness, trauma or stress situations(79). This coincidence can lead to severe and potentially life-threatening episodes of adrenal crisis. A case-control study which included 2.4 million people treated with GC therapy showed a 3.4 fold higher risk of AI per course of treatment year. Even inhalational GC therapy longer than ninety days showed an OR of 3.4 to develop AI, and this effect was dose related(80).

2.3.11. GC related bone disease

2.3.11.1. Osteoporosis (OP)

GCs stimulate on the one hand the osteoclast activity which leads to a reduced bone formation and on the other hand GCs potentially suppress osteoblastic activity in the bone marrow(81). In a meta-analysis which included more than 80 trials in adult patients, GC therapy was highly associated with significant decrease of bone mass, evaluated by bone mass densitometry (BMD) and adversely affected risk of bone fracture after 3 to 6 months of treatment with low dose GCs (prednisolone >5 mg/day or equivalent); Interestingly this gain of risk presented independently from patient age, gender and underlying disease(82). Even children face an elevated risk to develop rapid bone loss and fractures related to long-term GC therapy quantified with a hazard ratio of 1.3 in the cited publication(83). However, children with GC therapy in the history were not exposed to a higher fracture risk(84), in contrast to adults which previously followed a GC therapy(85).

2.3.11.2. Osteonecrosis

Osteonecrosis (ON) is defined by a localized destruction of an, often joint related bone area as the hip joint and is characterized by a decline of bone tissue which is related to an insufficient vascular supply, leading to infarction and necrosis of the affected area, and GC therapy is the main risk factor to develop ON(86). Risk for development of osteonecrosis is observed with 9-40% in adult patients with systemic or local intra-articular GC therapy. It occurs frequently in combination, but also without concomitant deficiency of bone mass quality(87). Also in children with hematological diseases, ON was highly associated with GC therapy(88). Presence of ON is frequently misdiagnosed and considered as lumbar pain syndrome or osteoarthritis(89).

2.4. Steroid-induced hyperglycemia

2.4.1. Definition

Steroid-induced hyperglycemia (SIHG), also called steroid-induced diabetes, is defined as an abnormal elevation of blood glucose in association to the exposure of GCs in patients with or without a preexisting history of diabetes mellitus.

Diagnostic criteria are in line to the diagnosis of the common diabetes mellitus type 2 (DM2), formulated by the American Diabetes Association (ADA)(90) as following circumstances:

- Fasting venous plasma glucose ≥ 126 mg/dl
- 2 h venous plasma glucose post 75 g oral glucose tolerance test (OGTT) ≥ 200 mg/dl
- Glycated hemoglobin 1c (HbA_{1c}) $\geq 6.5\%$ (≥ 48 mmol/mol)
- Random plasma glucose ≥ 200 mg/dl

2.4.2. Epidemiology

GCs are the main drug induced causer for steroid-induced hyperglycemia (SIHG)(24, 91). GCs induce exacerbation of hyperglycemia both in patients with previously diagnosed diabetes mellitus (DM) as well as in patients without known impairments in glucose

metabolism(92, 93). Incidence of hyperglycemia in patients receiving GC therapy without preexistent DM is estimated with 34-56%(17, 94, 95), glucose levels increase up to 68% compared to baseline prior to GC therapy (17, 96). Relative risk to develop SIHG is numbered with an OR of 1.36-2.31 accordable to a number needed to harm (NNH) reaching from 16-41 for 1-3 years of GC exposure(97, 98). Mainly systemic GC therapy showed diabetogenic potential while in patients using topic (eye drops, crèmes), or inhalational agents no clear or at least no considerable risk association could be elucidated. Even infrequent GC injections were not substantially connected to a higher risk to develop SIHG(99-101). The most prominent risk factors to develop SIHG are dose and type of steroid(102, 103), duration of GC treatment(17), a continuous GC regimen (OR: 2.0)(94), HbA_{1c}, body mass index (BMI)(OR2.15)(98, 104, 105) and advanced age(106). Additionally special population groups were identified to have a greater risk of SIHG as those with DM in family history, gestational diabetes, abnormal fasting glucose and impaired glucose tolerance(103, 107) prior to initiation to GC therapy. In hospitalized non diabetic patients who received potent doses of steroids, incidence of at least one hyperglycemic value was 86% and probability of showing an elevated fasting plasma glucose (>140 mg/dl in this study), was 48%(108).

2.4.3. Pathophysiologic mechanisms

The first theories on the diabetogenic effect of steroids relied on the GC promoting effect on insulin resistance on the basis of the liver, the skeletal muscle and the adipose tissue(25, 109-111).

2.4.3.1. Liver

The liver is a key organ in regulating metabolic processes utilizing its endocrine, nervous, autonomic and metabolic features.

Physiologically the liver maintains normoglycemia in fasting conditions by glucose production gained from gluconeogenesis and glycogenolysis. Hepatic glucose provision is inhibited in response to carbohydrate intake-dependent insulin decrease. Conversely, counter regulatory hormones as cortisol, epinephrine or glucagon which are secreted in acute stress situations or hypoglycemia, stimulate the liver for more production of glucose(112). Some experimental studies(113-116), but not all of them(113, 117, 118)

showed a GC induced influence on increase of endogenous glucose production (EGP). This increase seems to be rather related to gluconeogenesis than to glycogenolytic effects(119). Main promoter of GC induced gluconeogenesis are key enzymes as phosphoenolpyruvate carboxykinase (PEPCK)(120, 121) and glucose-6-phosphatase(122), which might have an influence on gene receptor response. An alternate theory indicates that gluconeogenesis is intensified by breakdown of peripheral protein and fat reservoirs(123). However, EGP is intensively decreased by action of insulin, especially notable in hyperinsulinemic clamp investigations in which, dependent on insulin amount, EGP is demonstratively suppressed up to his halves(114-116).

2.4.3.2. Skeletal muscle

Many scientific approaches were established which might declare the hyperglycemic potential of GC therapy based on a muscle tissue problem.

Skeletal muscle is the most prominent stage where postprandial insulin-stimulated glucose disposal takes place(124). By binding to its membrane-bound receptor, insulin promotes glucose uptake, glucose oxidation and glycogen synthesis by phosphorylation of several proteins, usually referred to as the insulin-signaling cascade(125). Cortisol was identified to be a blocking factor in the muscular glucose disposal, this theory hardens the opinion that GCs lead to an insulin resistance of muscle tissue(114, 126-128). Evidently, GC therapy leads to atrophic loss of muscle tissue and overall amount of muscle proportion of an individual(129), however there exist more cellular funded theories that not only the loss of quantitative muscular mass but also pathways promoting for insulin signaling are disturbed by GC exposure(130). These explanations take their offspring from theories which say that GCs lead to increase of nonesterified fatty acids (NEFA) and elevation of amino acids due to suppression of adipose tissue lipolysis, resulting in inhibition of insulin-stimulated muscular glucose uptake(131, 132). Additionally data exists that GCs directly affect insulin-induced recruitment of capillaries in skeletal muscle which might contribute to a decrease of insulin-mediated uptake of glucose(133, 134).

2.4.3.3. Adipose tissue

Endogenous or exogenous GC excess enhances fat deposition in the visceral compartment on the costs of subcutaneous adipose tissue (SAT) and leads to liver fat accumulation.

Additionally to the alteration of fat distribution also function of fat seems to be influenced by the fact that fasting lipolysis is accelerated(135). This lipolysis issue can be explained by elevated activity of the key lipolytic enzymes adipose triglyceride lipase (ATGL) and the hormone sensitive lipase (HSL) which increase plasma NEFA levels who then lead to hyperglycemia by impairment of muscle and liver insulin sensitivity(28). Furthermore tissue lipolysis is inhibited by insulin and triglyceride uptake becomes stimulated, shown in a hyperinsulinemic clamp study, in which GC treatment inhibited insulin-mediated suppression of lipolysis leading to higher plasma NEFA(114). The impact of adipose tissue disorders on hyperglycemia is also justified by the theory that fat-cells influence glucose tolerance by an interplay with the liver and skeletal muscle by secreting so called adipocytokines, and their variations showed to be diabetogenic impactors(136).

2.4.3.4. Pancreatic cells

2.4.3.4.1. Pancreatic β -cells

The pancreatic β -cell plays a crucial role in the modern declarations of diabetes etiology. The β -cell dysfunction (BCD) was identified as a main component in the development of DM2(137). The BCD arises as a result of an above the ordinary workload on the β -cell to produce insulin, which is caused by supernutrition, obesity and concomitant insulin resistance. Usually healthy β -cells adapt on this situation by enhancing insulin production to maintain normoglycemia(138). However β -cell reserves are limited and chronic overstimulation long-termly damages the islet cells substantially which leads to hyperglycemia, compatible with DM2. GC therapy revealed to be an inhibiting promoter of glucose-stimulated insulin secretion by influencing β -cell depolarization and insulin exocytosis as well as leading to impairment of intracellular insulin production and β -cell apoptosis(139, 140). Usually postprandial insulin secretion has two peaks, one high peak directly few minutes after food intake (insulin from cell storage is released) and the other peak more delayed (this insulin is newly produced by the prandial stimulus) in order to maintain euglycemia. Patients with prediabetes or impaired glucose tolerance pathophysiologically lose especially height and sooner presence of this first peak, therefore the organism takes longer to neutralize postprandial glycemia. And this was also shown for healthy subjects who received prednisolone in a hyperglycemic clamp study. In an extended study, in which healthy patients received GCs more extended (2 weeks), subjects

presented with higher fasting glucose, but showed improved β -cell response. This finding was explained as compensation mechanism which remained unfavorable(141).

2.4.3.4.2. Pancreatic α -cells

α -cells, as direct regulatory counterpart to β -cells, produce glucagon. Glucagon stimulates hepatic glucose synthesis by promoting gluconeogenesis and glycogenolysis. Together with insulin resistance and β -cell-dysfunction the impaired suppression of glucagon is involved in the development of DM2. In a postprandial state glucagon levels are usually decreased to a minimum, because there are no requirements of endogenous glucose production when carbohydrates were delivered to the organism. However this regulation is disordered in patients with DM2 and this circumstance leads to both fasting and postprandial hyperglycemia(142). GC therapy also impacts on serum glucagon levels in healthy subjects(143), but obviously this impact was only observed in subjects receiving higher doses (>30 mg prednisolone daily)(114).

2.4.3.5. The gut-islet axis

The most popular incretin hormones are glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are secreted in the ileum, caecum and duodenum. Physiologically incretin hormones are released after food intake in a glucose dependent manner and have an antihyperglycemic effect by stimulating meal-dependent insulin response. Additionally GLP1 decelerate gastric emptying which leads to promotion of satiety, reduction of appetite and loss of weight. Incretin hormones might also directly affect the hunger center in the brain. Exogenously administered GLP1 immediately induces pancreatic insulin secretion but interestingly only on the assumption that glucose levels are elevated(144). GLP1 has a very short half-life since it is cleaved by the enzyme dipeptidyl peptidase-4 (DPP-4). The utilization of incretin based therapeutics (GLP-1 receptor agonists (GLP1-RA) and DPP4 inhibitors (DPP4i)) have emerged in the last decades to be an established treatment of choice in patients with DM2 without having hypoglycemic potential. In spite of this there is only little information about the effect of incretin based therapy on SIHG. However, in mice studies a single dose of a GLP1 Agonist (exenatide) improved glucose intolerance and insulin resistance(145) and GC treatment led to a significant reduction of GLP1 levels(146). In healthy human two week treatment with

GCs did not affect glycemia after meal tests, but interestingly a less glucose-stimulated insulin secretion could be observed(147, 148), which, in my point of view, is led back to the alternative compensative glucose lowering mechanisms which exist in human individuals. Effects of therapeutic administration of GLP1-RA and DPP4i are described in more detail in chapter 2.5.3.

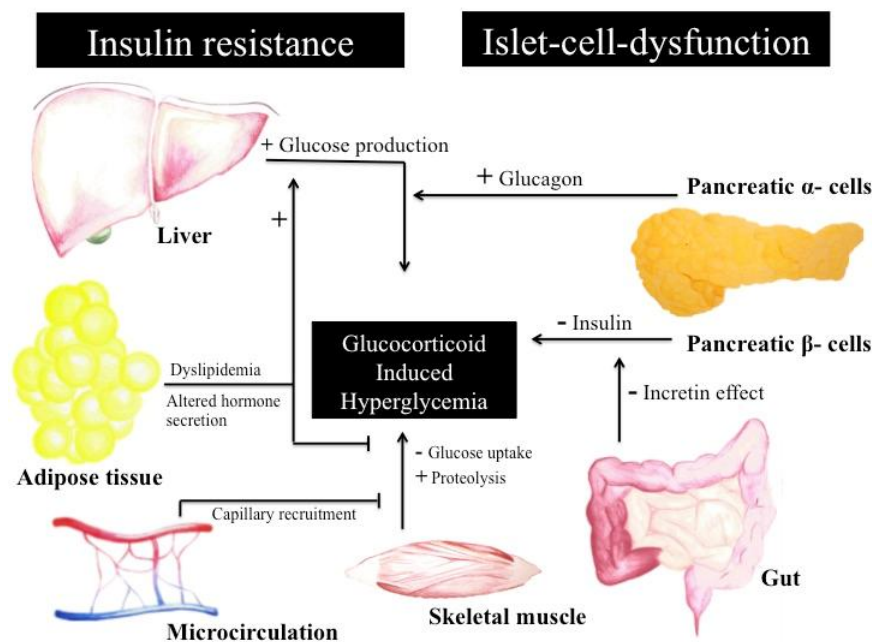


Figure 1 Mechanisms of glucocorticoid induced hyperglycemia Modified from (22) with permission from the first author Dr. van Raalte and designed by Magdalena Holzgruber.

2.5. Management of SIHG

Primary aim in the general treatment of SIHG does not differ from that one in patients with T2DM. Indicating that patients with SIHG have individual personal and therapeutical requirements, different epidemiologic characteristics, different health conditions and comorbidities, a unique recommendation for the best possible treatment cannot be implemented.

However individual therapy objectives include the avoidance of hypo-and hyperglycemia, freedom from glucose related symptoms and prevention of diabetes related late complications as CVD, diabetic microangiopathy or peripheral neuropathy.

By the extensive effects of GCs impacting on hyperglycemia, as indicated in chapter 2.3., many theories to identify the most effective and valuable therapy have been established. However, there is only little evidence and especially no general valid treatment guideline for SIHG.

2.5.1. Screening for SIHG

Recommendations for proactive screening before and short after initiation of GC therapy exist, however these do not have a high priority especially in non-endocrinological or internal departments.

In the beginning all patients should be advised about the general indication and potential side effect spectrum of GCs. Patients should draw their attention for hyperglycemic symptoms as polyuria, polydipsia or unexplainable weight loss. Following the optimum, all patients should receive determination of fasting and arbitrary plasma glucose, HbA_{1c} and OGTT prior to the start of GC therapy(149).

In hospitalized patients prescription of a 4-point glucose profile (measurement before the meals and bedtime) should be recommended from the day on GC therapy was established. In hospitalized as well as in patients in the outpatient clinic, a safety measurement which should not be influenced by meal intake should be arranged 8 hours after intake of the GC agent if short acting GC as prednisolone were prescribed(150). The Canadian Diabetes Association (CDA) guidelines 2013 recommend continuation of frequent glucose profiling for 48 hours in non-type 2 diabetic patients with initialized GC therapy(151). In patients who underwent transplantation, who are exposed to a lifelong immunosuppressant therapy, including GCs, weekly monitoring for four weeks and after that in an interval of 3, 6 and 12 months should be suggested(152). If an arbitrary value rises above 200 mg/dl, frequency of testing should be intensified to four times daily. If capillary glucose is consistently higher than 200 mg/dl (for example two times daily) the indicated reference recommends start of sulfonylurea (SU) therapy, however I will leave this recommendation hanging because we know the side effect profile of SU and we cannot generalize this treatment algorithm for all patients(153).

A rough estimation of general target glucose recommends preprandial values of 70-130 mg/dl and postprandial glucose <180 mg/dl, as well as HbA_{1c} lower 7%. These target areas

do not alter to the therapy targets in patients with DM2 and certainly cannot be operated for all patients with SIHG or any other kind of diabetic disease(154).

2.6. Therapeutic options for SIHG

2.6.1. Life style modification

Modification of lifestyle as primary procedure in the management of DM2 might also be a supportive measure in the treatment and prophylaxis of SIHG in consideration that overweight and malnourished individuals tend to develop impaired glucose metabolism anyway(149).

2.6.2. Oral antidiabetic agents

There is only little evidence on the efficacy of oral antidiabetic drugs (OAD) to treat corticoid-induced diabetes. However, in early stages of SIHG, OAD therapy in combination with life style modification has shown to be a therapeutic possibility(92, 152). Majority of OADs have only a little capacity to adopt to major changes in glycemia, furthermore doubts about the efficacy of OADs fulfilling the requirements of the complexity of SIHG therapy, exist(103).

2.6.2.1. Metformin

Metformin is, at least in patients with DM2, designated to inhibit gluconeogenesis, enhance peripheral glucose uptake and by this reducing insulin resistance, and therefore might theoretically be also effective in patients with SIHG. However, there is only sparse data underlining the efficacy of metformin in the treatment of SIHG. I could identify one single study which tested the use of metformin in 17 children with acute lymphoblastic leukemia and steroid related hyperglycemia. Results describe efficacy only in a small percentage of patients but a safe use(155). However, aside from the sparse basis of data, it is considerable that metformin can lead to hypoxic disorders and renal impairments what is another limiting factor for use especially in severely ill patients suffering from SIHG(95, 98).

2.6.2.2. Secretagogues

Sulfonylurea (SU), or relatives, are known to stimulate glucose independent insulin release from pancreatic β -cells immediately after administration of the agent and possess also the availability to decrease peripheral glucose uptake(156). Nevertheless SU is the OAD with the greatest hypoglycemic potential and this circumstance is especially then dangerous when it is prolonged used and not administered in combination with the recommended GC, for example when GC therapy is tapered but SU therapy is not adopted accordingly(98). Therefore shorter acting secretagogues (meglitinide analogues), which should be administered simultaneously to GC agent, might be beneficial options to treat the GC related decrease of glucose levels(157). However also in context to use of SU in SIHG evidence is rare and inconclusive.

2.6.2.3. Thiazolidinediones (TZD)

TZD agents as rosiglitazone and pioglitazone activate the peroxisom proliferator-activated receptor gamma (PPAR γ) in the cellular nucleus. By this, expression of genes related to the glucose and lipid metabolism is increased. This mechanism leads to an enhanced intracellular glucose uptake, especially in muscle and adipose tissue, while having only a little effect on insulin secretion – by this TZD are also known as insulin sensitizer. As it is in other OADs there is only sparse data on TZD confirming a beneficial effect on SIHG. Some publications describe a possible supporting effect in combination to alternative antidiabetic drugs as insulin in patients which underwent transplantation(158, 159). However, there is still a justified concern about the broad spectrum of TZD in the treatment of SIHG by the fact that TZD are potentially responsible for fractures, edema and congestive heart failure and these undesirable side effects are connected to GC therapy itself anyway(93, 98, 160).

2.6.2.4. Incretin mimetics

Dipeptidyl peptidase inhibitors (DPP4-i) and Glucagon-like-peptide-1 receptor-a gonists (GLP1-RA) stimulate postprandial insulin and suppress postprandial glucagon release. Further effects are the slowing of gastric emptying, reduction of appetite and possibly also the suppression of the central hunger system which consecutively induces weight loss(161). In mice studies a single shot of the GLP1-RA exenatide was able to improve

glucose intolerance and insulin resistance(145). A placebo controlled trial, performed in healthy men receiving different amounts of prednisolone or placebo, showed a preventive effect to develop post-exposure hyperglycemia based on the decrease of glucagon levels and decelerating of gastric emptying after OGTT. This result brought the authors to the recommendation to consider GLP1-RA as prophylaxis to SIHG(162). In an unrandomized study of 19 patients with SIHG the DPP4-I Sitagliptin was able to decrease fasting plasma glucose and HbA_{1c} significantly during a 6 months treatment(163).

2.6.2.5. Sodium-glucose linked transporter-2 inhibitors (SGLT2-inhibitors)

SGLT2-inhibitors (gliflozines) are novel group of OADs which gain their glucose lowering effect by inhibiting glucose reabsorption in the proximal tubule of the renal nephron. This inhibitory effect leads to a desirable glucosuria and improvement of plasma glucose(164). To my knowledge, there are no published articles focusing on SGLT2-inhibitors in the treatment of SIHG available yet.

2.6.3. Insulin therapy in the treatment of SIHG

Insulin treatment is the most tested and recommended option in patients with SIHG. In general it is suggested to initiate insulin therapy in patients with persistent hyperglycemia >200 mg/dl with or without OADs. GC associated insulin resistance initially requires large amounts of insulin until the moment the resistance barrier is broken through and glycemic control is on a stable level(165).

Kind of insulin therapy has to be chosen and adjusted on the one hand to individual patient's characteristics as BMI, renal function, age, desired target area; secondly it is essential to take quality of glucose disorder, meaning the time, onset, duration and severity of GC connected elevation of glucose excursion, into account. Development of more reliable kinetic profiles of specific insulins has permitted and facilitated different treatment strategies.

2.6.3.1. Prandial insulin therapy (PIT)

GC agents with a short-acting corticoid effect (as prednisone, prednisolone, hydrocortone) show a fast onset and a relatively short time of duration, as well as from the corticoid but also from the glucose elevating side. Serum glucose levels decrease directly after administration of GC agent which is explainable by the defective postprandial insulin secretion(166). For this reason, a PIT can be recommended in patients with single uses of short acting GCs once or twice daily(166). Insulins of choice in this manner are short

acting regular insulins or insulin analogues. Amount of dose is dependent on the power of GC related glucose decrease, scheduled meals and GC dose(17, 100). A simplified method to initialize PIT turned out to be the calculation of 0.1 insulin units (IU)/kilogram (kg) bodyweight (BW). In this context, PIT can be intensified by including insulin corrections in cases of higher initial preprandial values or remaining postprandial hyperglycemia. Thus, this intensification requires pre/postprandial self monitoring of blood glucose (SMBG). In such cases schematic counting of 0.04 IU/kg for preprandial values from 200-300 mg/dl or 0.08 IU/kg for values >300 mg/dl can be added to the conventional insulin dose. Additionally applied carbohydrates could also be covered with bolus insulin if required, implicating that a training on carb counting is necessary. Important to mention is that requirements of insulin are GC dose-dependent, hence reduction of GC is always connected to an improvement of glycemia. In this relationship it could be recommended to proportionally decrease insulin dose with decrease of GC dose, e.g. if a patient is on methylprednisolone (Urbason®) 20 mg in the morning and therefore injects 8 units of bolus insulin, he should, in case of tapering Urbason to 10 mg, also halve the dose of insulin, to 4 IU for this example.

2.6.3.2. Basal insulin therapy

Introduction of a basal insulin is recommended when fasting glucose values trespass a threshold of 200 mg/dl. This is often the case in patients with a known or unknown trend towards impaired glucose tolerance or prediabetes prior to GC initiation. Biggest evidence in context to basal insulin in SIHG exists for the utilization of intermediate acting basal insulins as neutral Protamin Hagedorn (NPH)- Insulin or also insulin detemir(98, 167-170). On the one hand these insulins have a shorter profile of basal insulin effect (estimated 12 hours(171)) and therefore it might be preferable in patients where extended GC action and longer disposition of GC induced hyperglycemia is present but glucose normalizes in the afternoon/evening and during nighttime. On the other hand the shorter basal insulin effect allows better regulation in terms of different insulin requirements during day-and eventually during night time, implicating that patients could inject basal insulin twice daily, which is not recommended in patients using longer acting basal insulins as insulin glargine or insulin degludec.

However there are recent publications which showed no general benefit of NPH in contrast to insulin glargine. Radhakutty performed a randomized trial in which they included 50 patients with a single morning dose of prednisolone ≥ 20 mg. Patients received even NPH

insulin or glargine insulin in the morning in combination with prandial insulin to cover meals or correct premeal hyperglycemia. 50% of the calculated total daily dose were NPH/glargine. In the NPH group distribution of bolus insulin (insulin aspart) was 20, 40, 40% administered before breakfast, before lunch and dinner. In the glargine group bolus insulin was divided in homogeneously thirds. In summary there were no differences in efficacy or safety between the both treatment options(172). Dinthal et al. published similar results and additionally could see less of required insulin to establish proper glycemic control when taking NPH insulin(173).

Another finding was generated by Brady et al, who tested a paper based algorithm (sliding scale) for basal-bolus insulin in 23 patients (91% were diabetic previously to GC therapy) with cycles of chemotherapy. They showed an improvement of glycemia during the different cycles of chemotherapy by using an algorithm which calculated 63-77% of prandial and 23-37% of basal insulin. Therefore, and also because of a little incidence of hypoglycemia they postulate to initiate insulin therapy with 1-1.2 IU/kg/day which is quite higher as in other recommendations. Furthermore they recommend distribution of basal to bolus insulin with a proportion of 25 and 75% which is also extraordinary in comparison to other trials(174).

A suggestion to initiate basal insulin is a calculation of 0.1 IU/kg injected in the morning when the GC agent is administered and eventually in the evening if fasting glucose values remain too high. If hyperglycemia levels remain >300 mg/dl despite bolus corrections, 0.04 IU/kg at glucose ranges between 300-400 mg/dl and 0.05 IU/kg >400 mg/dl can be added(157). In cases of nocturnal hypoglycemia a longer acting basal insulin with a flatter effective curve may be an option(175), however this was not explicitly proven in patients with SIHG. Especially in patients with transient but significant hyperglycemia provoked by GCs and foreseeable quick cessation of GC therapy premixed insulins which contain basal- and bolus insulin within only one pen, can be prescribed.

Tamez-Pérez et al. developed a 2015 published schematic algorithm in the guidance of insulin therapy in patients with SIHG. They recommend application of NPH insulin for patients who administer a single daily dose of GC therapy. The insulin should be applied at the time point the GC is ingested, generally in the morning with a starting dose of 0.4 IU/kg. If multiple GC doses are intended it is likely that NPH insulin is not enough to acquire satisfying glycemic control. In such case dose can be divided to 30% basal and

70% prandial insulin(175). When using longer acting GC therapy as dexamethasone, longer acting basal insulins might be more effective(98). In patients who were already on insulin therapy prior to GC implementation dose should be increased by 20%. Another approach in preinsulized patients is to initiate a new dose with 0.7IU/kg per day. In patients with high or uncontrollable, difficult to predict hyperglycemia (especially patients with intravenous GC pulse therapy), continuous insulin infusion by insulin pump or intravenous options might be helpful(92, 176).

The insulin dose should be adapted every 2-3 days with in-or decreases of 20% of insulin dose. Furthermore insulin doses should be adjusted based on changes of GC dose to prevent dose related sudden alterations of glycemia(175). Percentage of insulin amount should be adjusted in correspondence with changes of GC therapy dose, meaning that for a bisection of steroid dose, insulin dose should be reduced/increased accordingly(103).

2.7. GvHD

2.7.1. Definition of GvHD

Acute and chronic graft-versus-host-disease (GvHD) is a frequent and life-threatening complication occurring in patients who underwent allogeneic HSCT. GvHD is characterized by an attack of donor lymphocytes (graft) which is leading to systemic inflammation and necrosis of several recipient organ systems (mainly skin, liver, gastrointestinal tract). GvHD can be separated into an acute and a chronic form. While acute GvHD occurs within the first 100 days after HSCT, chronic GvHD appears more delayed. Other, more detailed, definitions include:

- *Classic acute GvHD*: Presentation within 100 days after HSCT and appearance of typical features of acute GvHD.
- *Persistent, recurrent, late onset acute GvHD*: GvHD appears later than 100 days after HSCT, but presents with diagnostic and distinctive features of acute GvHD.
- *Classic chronic GvHD*: This type appears after 100 days post HSCT and fulfills clinical features of a chronic disease.
- *Overlap- Syndrome*: Cases present at any time with showing criteria for both, acute and chronic features (acute on chronic GvHD)(177).

2.7.2 Epidemiology of GvHD

Despite GvHD prophylaxis is integrated in all therapy regimens, GvHD incidence rates range from 9-80% in patients who receive HSCT from a genotypically HLA identical donor. Acute GvHD usually develops 2-3 weeks after transplantation, in patients who live longer than 100 days after HSCT, chronic GvHD occurs in 30-70%(177, 178).

2.7.3. Risk factors for GvHD

The most prominent risk factors for the development of GvHD are

- the degree of HLA quality (HLA mismatch or unrelated donor)
- gender disparity between donor and recipient
- intensity of the conditioning regimen
- quality of prophylaxis
- source of graft (peripheral blood or bone marrow greater than umbilical cord blood)

Less potent risk factors which could be identified were age of the host, status of infection with cytomegalovirus (CMV) or Epstein-Barr Virus (EBV) seropositivity, presence of a sterile environment (e.g. Helicobacter pylori infection) and particular HLA haplotypes(57, 179-182).

The risk and also the severity of GvHD is associated to pre-transplant comorbidity status, which can be determined by using the hematopoietic cell transplantation-specific comorbidity index (HCT-CI), also called Sorror-score. In a study of almost 3.000 graft recipients, probability to develop severe GvHD was directly associated to HCT-CI score with percentages from 13 to 24 in patients with a score of 0 versus >5(183).

2.7.4. Pathogenesis of GvHD

Acute GvHD develops when T-cells of the donor recognize host tissue as foreign and a cascade of inflammation is instituted. These mechanisms are based on HLA incompatibilities by recognition of histocompatibility antigens from donor's T-cell receptors. The inflammatory processes which develop can be considered as a distortion of the cellular responses to viral and bacterial infections and present mainly on liver, skin and

the upper and lower gastrointestinal tract(184). Ferrara et al. graduated pathogenesis of GvHD to three phases.

1st phase: Damage to recipient tissue by inflammation caused by preparative chemo- or radiatio- therapy regimen.

2nd phase: Donor antigen-presenting cells and inflammatory cytokines induce activation of donor-derived T-lymphocytes, which develop to effector cells.

3rd phase: In the effector phase, activated donor T-cells mediate cytotoxicity against target recipient cells by ligand interactions and production of cytokines (e.g. TNF- α) which lead to further activation of inflammatory cytokines as interleukin (IL)-1, IL-6, IL-10, IL-12. This allogeneic interactions lead to tissue damage compatibly to GvHD(185, 186).

2.7.5. Clinical and histological manifestations of GvHD

Principal targets which are affected by GvHD-reactions are liver, skin and gastrointestinal tract (GIT). However in most cases, GvHD appears multifocal presenting with combinations of these. In about 3 of 4 cases the skin and the GIT are affected. Percentage of liver involvement is numbered with 44%(187).

2.7.5.1. GvHD of the skin

In the majority of GvHD patients the initial clinical manifestation of acute GvHD is a maculopapular rash of the neck, shoulders, palms or foot soles. This appearance is commonly occurring during the time of the white blood cell engraftment. It usually presents with redness similar to a sunburn, pruritus or pain and potentially spreads over the integument, eventually becoming confluent.

In order to exclude other causes (as allergic exanthema or toxic skin damage), skin histology is required in which typical changes in dermal and epidermal layers can be observed(188). Typical histological signs include exocytosis of lymphocytes with satellite lymphocytes, including follicular involvement and dermal perivascular lymphocytic infiltration. Chronic cutaneous GvHD involvement also includes affection of oral mucosa, nail dystrophy or loss, lichen-type features, ocular sicca- syndrome and conjunctivitis and manifestations of muscle tissue presenting with fasciitis or myositis(189, 190).

The degree of acute cutaneous GvHD severity can be graded to 4 different stages upon visual objection:

- *Stage 1*: Maculopapular rash affecting less than 25% of body surface
- *Stage 2*: Maculopapular rash affecting 25 to 50% of the body area
- *Stage 3*: Generalized erythroderma
- *Stage 4*: Generalized erythroderma with bulla formation



Figure 2 Cutaneous manifestations of acute GvHD. A Grade 1, B Grade 2, C Grade 3, D Grade 4. Pictures purchased and permission to reproduce received from visualdx.com

A further classification of skin GvHD severity is based on histological features and is not outlined here.

2.7.5.2. GvHD of the gastrointestinal tract

GvHD of the GIT usually affects both, the lower and upper tract and commonly initially presents with abdominal pain, nausea, vomiting, and weight loss. However cardinal symptom and main criteria for diagnosis is the presence of diarrhea (with or without blood). By the fact that acute GvHD of the GIT occurring elsewhere in the GIT could be

diagnosed from endoscopically inconspicuous tissue from the rectum, rectal biopsy is mostly enough to histologically verify GvHD of the GIT. However, a negative result from rectal biopsy does not exclude presence of GIT- GvHD. Next, patients with clinical suspicion but negative rectal biopsy should undergo colonoscopy or gastroscopy(192, 193). The definite diagnosis requires histological confirmation, further grading of severity is conducted by quantifying the amount of diarrhea:

- *Stage 1*: Diarrhea 500-1000 ml/day
- *Stage 2*: Diarrhea 1000-1500 ml/day
- *Stage 3*: Diarrhea 1500-2000 ml/day
- *Stage 4*: Diarrhea >2000 ml/day OR severe abdominal pain or ileus

Diarrhea presents secretory and occurs independently from oral fluid intake. Concomitant gastrointestinal blood loss can potentially lead to severe hypovolemia and shock conditions, requiring blood transfusions. Severe cases can lead to ileus, which is also triggered by high opiate requirements the discomfort of GvHD is bringing along.

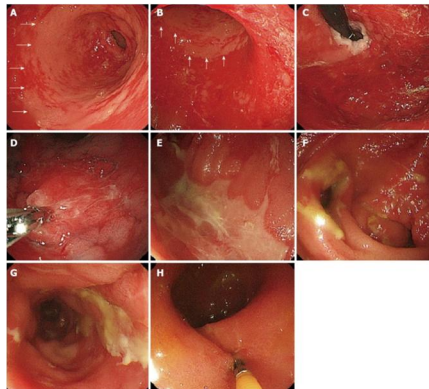


Figure 3 Upper and lower gastrointestinal endoscopic findings (194). Reproduced from (22) with open access permission to distribute and use for non commercial purposes (<https://www.wjgnet.com/1007-9327/full/v23/i27/4856.htm>).

2.7.5.3. GvHD of the liver

Hepatic GvHD involvement usually appears in patients with signs of skin or GIT- GvHD and rarely occurs alone(191). GvHD of the liver is diagnosed by an increase of liver function parameters (conjugated bilirubin and alkaline phosphatase). Liver GvHD can lead to painful hepatomegaly, pale stool and pruritus. Elevation of liver function parameters are not only correlated to GvHD but can also be a concomitant phenomenon of hepatic

(super)-infections, drug toxicity or obstructive syndromes. Thus, biopsy of the liver is recommended to confirm diagnosis if deemed necessary; severe predispositions to bleeding (low platelet counts or coagulation disorders) frequently prohibit conduction of liver biopsy.

To classify severity of liver GvHD total bilirubin levels are used:

- *Stage 1*: Bilirubin 2-3 mg/dl
- *Stage 2*: Bilirubin 3-6 mg/dl
- *Stage 3*: Bilirubin 6 to 15 mg/dl
- *Stage 4*: Bilirubin >15 mg/dl

2.7.5.4. Other organs affected by GvHD

In rare cases also the lungs, eyes, thymus, heart and kidneys can be affected by acute GvHD reactions.

2.7.6. Grading of acute GvHD

Except from the organ specific stage-classifications, general acute GvHD grading scores were established to classify GvHD for prognostic issues. The most prominent grading tools are the Glucksberg grade (1-4) and the International Bone and Marrow Transplant Registry (IBMTR) grading system.

The Glucksberg scale is taking severity of skin, GIT and liver GvHD in combination to patient's performance status into account. The performance status is determined by the Eastern Cooperative Oncology Group (ECOG) scale(195). Some authors use the Karnofsky index for determining performance status(196).

| The ECOG scoring system versus the Karnofsky scoring system | | | |
|--|---|--|-----------|
| ECOG/WHO | | Karnofsky score | |
| Fully active, no restrictions | 0 | Normal, no evidence of disease Able to perform normal activity with only minor symptoms | 100 90 |
| Restricted in strenuous activity Ambulatory, can carry out work | 1 | Normal activity with effort Able to care for self but unable to do normal activities | 80 70 |
| Ambulatory >50% of the time Capable of self-care Unable to work/usual activities | 2 | Requires occasional assistance, cares for most needs Requires considerable assistance | 60 50 |
| Ambulatory <50% of the time Capable of limited selfcare only | 3 | Disabled, requires special assistance Severely disabled | 40 30 |
| Disabled, no self-care Confined to bed or chair | 4 | Very sick, requires active support Moribund | 20 10 |

Table 3 Individual performance status assessed by the ECOG scale(195) and the Karnofsky score(196)

| Grade of GvHD | Degree of organ involvement |
|---------------|--|
| 1 | - Skin: 1 to 2 |
| 2 | - Skin: 1 to 3 - GIT and/or liver: 1 - Mild decrease in clinical performance |
| 3 | - Skin: 2 to 3 - GIT and/or liver: 1 - Moderate decrease in clinical performance |
| 4 | - Skin: 2 to 4 - GIT and/or liver: 2 to 4 - Extreme decrease in clinical performance |

Table 4 Glucksberg scale for Grading of acute GvHD.

2.7.7. Prophylaxis of GvHD

Aim of GvHD prophylaxis is the prevention of GvHD appearance. Intensity and duration of prophylaxis therapy is based on remission status of the underlying disease, age of the recipient and degree of HLA compatibility between recipient and donor. The most frequent operated GvHD prophylaxis regimen consists of a combination of calcineurin-inhibitors as cyclosporine A, or tacrolimus with methotrexate or mycophenolat(197).

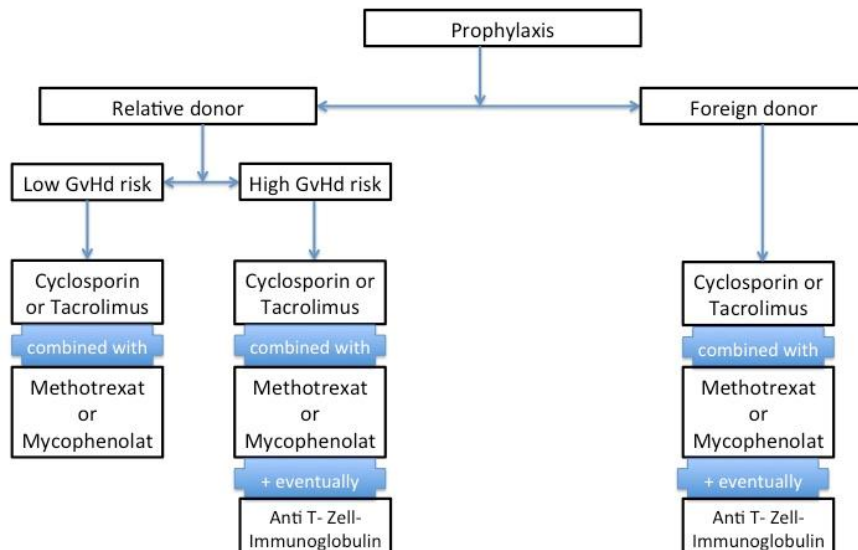


Figure 4 Schematic algorithm for GvHD prophylaxis according to (197)

2.7.8. Therapy of GvHD

Therapy of acute GvHD is based on the severity of GvHD. Diseases with grading higher Glucksberg 2 require systemic immunosuppressant therapy. First-line therapy includes

high-dose systemic corticosteroid therapy usually administered with 2 mg/kg body weight daily for 1 week. Patients with GIT GvHD additionally receive non-absorbable oral steroids (budesonide) in a dose of 9 mg/day. When patients do not respond to corticosteroids within 4 days or GvHD even deteriorates, a second-line therapy, mostly with mycophenolat, has to be additionally initiated. If patients do respond after 4 days, prednisolone monotherapy is continued until day 7 and then tapered by 20% every 5 days. Supplementary to specific GvHD therapy, supportive measures as fluid substitution, parenteral nutrition, anti-infective therapy, anti-mycotic therapy, analgesia and gastric protection are prescribed(198).

2.8. Aim of the study

Aim of this study was to focus on the impact of glycemic status to prognostic outcomes as overall mortality in patients who were suffering from acute GvHD(22).

2.9. Study design, parameters of interest, regulatory issues

We retrospectively collected data from medical records and the hospital electronic documentation program MEDOCS (Medical Documentation and Communication System). Hereby, many thanks to Dr. Melanie Stauber for careful collection of related data.

104 patients who received systemic steroids for histologically proven acute GvHD were included in this analysis. Data was obtained from GvHD patients, who were hospitalized at the Division of Hematology at the Medical University of Graz, for acute GvHD during the years 2004 and 2016. The study was registered and approved by the local Ethics Committee (Registration number 27-270 ex 14/15).

For each subject, glucose values were obtained from regular blood glucose measurements (most of them finger prick measurements, partly plasma glucose) during in- and outpatient setting. Patients were followed up until their last contact or death. Additionally disease-related information was collected including time of stem cell transplantation, underlying disease and further comorbidities, hematopoietic cell transplantation-specific comorbidity index score (HCT-CI score) and GvHD-related (organ affection, Glucksberg-grading...) details.

2.9.1. Statistical analysis

Baseline characteristics were illustrated and calculated using descriptive statistics including mean, median and standard deviation.

The main variable of interest in this retrospective study was the influence of hyperglycemia on overall survival (OS) and non-relapse mortality (NRM), defined as the duration of acute GvHD onset until death from any reason (for OS) or until death from non-relapse. The main covariates or independent variables were mean, median and maximum of the longitudinal glucose values.

In order to classify patients to glucose categories, tertiles were created related to the summary glucose parameters and illustrated graphically by survival curves. Furthermore, early hyperglycemia during the first three days after GvHD onset, which was defined as mean glucose >125 mg/dl was used as binary variable and was also used as covariate separately for further analyses.

For the OS analysis Kaplan-Meier survival plots were used to illustrate a relation between glucose tertiles and OS. Statistical significance was calculated with log-rank testing and Cox proportional hazard models. For NRM analysis, we used the Fine and Gray prediction model to account for the presence of competing risks as death due to relapse. For identifying independence of specific factors as age, BMI, HLA identity, steroid dose, response to steroids, disease risk index, donor type (sibling versus nonsibling) we performed adjustment for indicated factors.

Statistical analyses were executed using either GraphPad Prism software version 7.0 or R software version 3.0.3.

2.9.2. Material and methods

104 patients (46 female) were enrolled in this analysis. Main inclusion criteria was a history of stem cell transplantation and related acute GvHD, which was histologically confirmed. 2 of the patients had a pre-existing diabetes. Description of baseline characteristics are illustrated in Table 5.

| | | |
|--------------------------------------|-------------------------------------|------------|
| Gender | female | 48 (46.2%) |
| | male | 56 (53.8%) |
| Age (in years) | <30 | 8 (7.7%) |
| | 30-39 | 17 (16.4%) |
| | 40-49 | 25 (24.0%) |
| | 50-59 | 36 (34.6%) |
| | ≥60 | 18 (17.3%) |
| Body Mass Index (kg/m ²) | <19 | 4 (3.8%) |
| | 19-25 | 60 (57.7%) |
| | 25-30 | 35 (33.7%) |
| | ≥30 | 5 (4.8%) |
| Underlying disease | Acute myeloid leukemia | 60 (57.7%) |
| | Acute lymphoblastic leukemia | 15 (14.4%) |
| | Myelodysplastic syndrome | 7 (7.6%) |
| | Chronic myeloproliferative disorder | 5 (4.8%) |
| | Others | 17 (16.4%) |
| Disease risk index | Low/intermediate | 65 (62.5%) |
| | High/very high | 39 (37.5%) |
| Comorbidity (HCT-CI) | HCT CI 0 | 55 (52.9%) |
| | HCT CI ≥ 1 | 49 (47.1%) |
| Conditioning regimen | Myeloablative | 40 (38.5%) |
| | non-myeloablative | 64 (61.5%) |
| GvHD prophylaxis | Ciclosporin/Mycophenolate | 58 (55.8%) |
| | Ciclosporin/Metotrexate | 40 (38.5%) |
| | Other | 6 (5.7%) |
| Antithymocyte globulin for | Yes | 69 (66.3%) |
| | No | 35 (33.7%) |
| Donor | Cord blood | 11 (10.6%) |
| | Unrelated | 64 (61.5%) |
| | HLA-identical sibling | 27 (26.0%) |
| | Haploidentical | 2 (1.9%) |
| HLA match | Identical | 65 (62.5%) |
| | Mismatch | 39 (37.5%) |
| Gender-matching | Same gender | 50 (48.1%) |
| | Male donor, female recipient | 23 (22.1%) |
| | Female donor, male recipient | 25 (24.0%) |
| | Not known | 6 (5.8%) |

Table 5 Baseline characteristics

Median duration from transplantation to onset of GvHD was 25 days, 10 patients suffered from late onset GvHD, diagnosed later than 100 days after HSCT. 46 patients presented with GvHD stage 2, while 58 patients had grade 3 or grade 4. More than 2 thirds of patients received methylprednisolone 2 mg/kg/day as first line therapy. The median duration of steroid treatment was 60 days (10-241 days). First line therapy, implemented as therapy with corticosteroids was initially effective in 57.7%. A median of 2 salvage immunosuppressant therapies were necessary in 45 patients, either because of a lack of response or due to reoccurrence of any GvHD during tapering of corticosteroids. 33 patients (31.7%) reached complete resolution of any GvHD sign, defined by disappearance of all GvHD symptoms and possibility to terminate GvHD specific therapy(22). Summarized GvHD characteristics are illustrated in table 6.

| | | |
|---|------------------------|-----------|
| Number of GvHD affected organs | 1 | 38 (36.5) |
| | 2 | 54 (51.9) |
| | 3 | 12 (11.6) |
| Number of patients with affected organ | Gastrointestinal tract | 82 (78.8) |
| | Skin | 78 (75.0) |
| | Liver | 17 (16.3) |
| Overall grading (Glucksberg score) | 2 | 46 (44.2) |
| | 3-4 | 58 (55.8) |
| Dosage of glucocorticoids, mg per kg body weight | <2 | 32 (30.8) |
| | 2 | 72 (69.2) |
| Response to first line corticosteroid treatment | Response | 60 (57.7) |
| | No response | 44 (42.3) |
| Number of additional immunosuppressant lines of therapy | 1 | 22 (21.2) |
| | 2 | 13 (12.5) |
| | ≥3 | 10 (9.6) |
| Outcome of GvHD at last follow-up | Resolved | 33 (31.7) |
| | Not resolved | 71 (68.3) |

Table 6 GvHD characteristics, numbers mean n (%)

2.10. Results

2.10.1. Hyperglycemia and outcome

The median follow up of the overall cohort was 35.7 months (IQR 19.5-94.4 months), the median OS was 8.0 months (95% CI 4.5-19.8 months). The probability to be alive within the first, second and fifth year of follow up was 44.9%, 34.8% and 31%, respectively. 36.5% of patients (n=38) were alive at last date of follow-up. For each patient, glucose values were collected during the overall follow up. The median number of available glucose parameters was 50 (4-513). Median, mean as well as maximum of glucose values were calculated for every patient. Mean glucose was 141.9 mg/dl (86.9-255.4 mg/dl), median glucose was 134.5 mg/dl (79-235 mg/dl) and maximum was 248 mg/dl (115-793 mg/dl).

Patients were divided to tertiles according to their glucose values during observation to evaluate the influence of hyperglycemia to OS. Increasing blood glucose was related to a higher mortality with a reduction of 5 and 10 months in patients who were allocated to categories 2 and 3, respectively ($p < 0.0001$ for trend), displayed by a Kaplan-Meier survival plot (Figure 5).

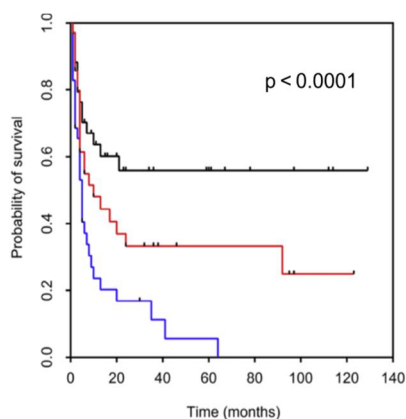


Figure 5 Probability of survival according to mean glucose levels during GC treatment. Tertile 1 (black line) contained patients with a mean glucose of 86.9-127.6 mg/dl, tertile 2 (red line) contained patients with a mean glucose of 128.3-164.7 mg/dl and tertile 3 (blue line) included patients with a mean glucose of 165.7-255.4 mg/dl. The p-value indicates a statistical significance for trend evaluated by a log-rank test. Reproduced from (22) with permission from the copyright holder.

Additionally we focused on whether patients required insulin therapy or not and we could demonstrate that patients who received insulin also faced a higher probability for mortality as displayed in figure 6.

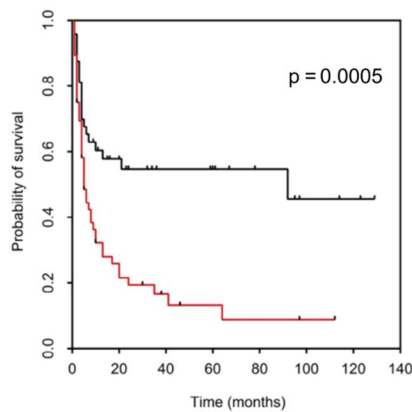


Figure 6 Kaplan Meier - survival curve indicating that patients treated with insulin (red line) faced a statistically significant higher risk for OS ($p < 0.0005$ is indicating a statistical significance for trend derived by log-rank testing). Reproduced from (22) with permission from the copyright holder.

The impact of hyperglycemia to influence survival was even similarly powerful after taking other parameters as age, BMI, donor type, HLA identity, response to GC and underlying disease into account in a multivariate model.

2.10.2. Impact of early hyperglycemia

As a next, we investigated whether hyperglycemia appearing during the first days after GvHD onset represents similar prognostic information than overall hyperglycemia. Therefore we extracted the glucose values during the first 3 days after GvHD diagnosis and initiation of GC therapy. We were able to observe a good correlation between the patients' 3 days glucose and overall glucose levels during the whole period of observation ($r=0.56$). We defined hyperglycemia as glucose mean level >125 mg/dl and we were able to demonstrate that hyperglycemic patients were exposed to significantly higher overall mortality than patients presenting with a mean glucose ≤ 125 mg/dl. Figure 7 illustrates the cumulative incidence of OS according to glycemic status during the first 3 days after initiation of GC therapy.

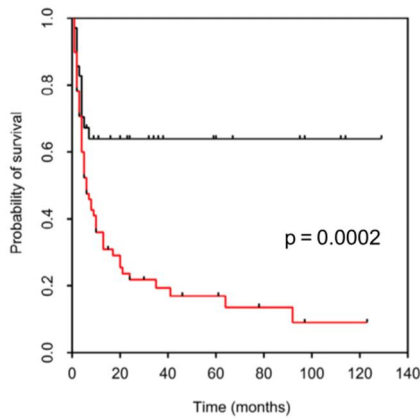


Figure 7 Probability of survival according to mean glucose levels during the first 3 days of GC treatment. Early hyperglycemia was defined as mean glucose > 125 mg/dl. Patients who were hyperglycemic (red line) had an enhanced risk of all-cause mortality (HR 2.9, $p=0.0002$) in contrast to patients who presented with a mean glucose lower or even 125 mg/dl (black line). Reproduced from (22) with permission from the copyright holder.

Additionally, patients who were hyperglycemic also faced a higher risk for non-relapse mortality (HR 2.88, $p=0.001$) but contrary and interestingly did not tend to have a higher risk of relapse of underlying disease ($p=0.85$). Figure 8 and 9 focus on the impact of hyperglycemia to non-relapse mortality (NRM) and relapse.

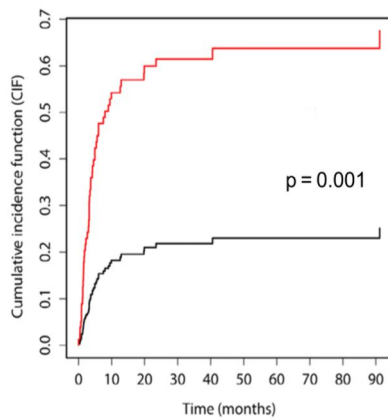


Figure 8 Impact of early hyperglycemia on incidence of NRM between hyperglycemic patients (red line) and patients with a mean glucose level ≤ 125 mg/dl (black line). The p-value indicates statistical significance for trend derived by log-rank testing. Reproduced from (22) with permission from the copyright holder.

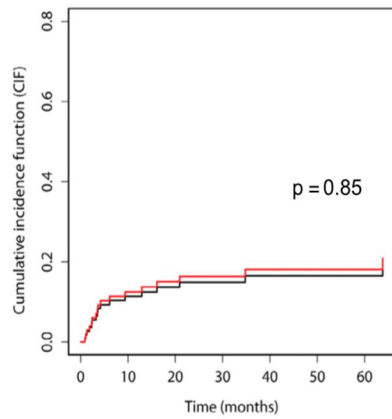


Figure 9 No difference in relapse risk according to hyperglycemic (red line) and non-hyperglycemic (black line) patients. Reproduced from (22) with permission from the copyright holder.

Reasons for NRM (42 in the hyperglycemic and 7 in the non-hyperglycemic cohort) were infection (45.2 vs. 42.9%), refractory GvHD (35.7 vs. 42.9%) and other causes (19.1 vs. 14.2%). In a multivariate analysis including the above mentioned parameters, early hyperglycemia was an independent and the most prominent risk factor for overall mortality (HR 2.49, $p=0.006$) and NRM (3.54, $p=0.001$), as illustrated in table 7 and 8. Interestingly, pre-transplant glucose levels were not associated to a higher mortality.

| Covariates | HR | 95% CI | | P Value |
|-----------------------------------|-------|--------|-------|---------|
| | | Lower | Upper | |
| Early hyperglycemia | 2.490 | 1.300 | 4.770 | .0060* |
| Age | 1.007 | .984 | 1.030 | .5562 |
| HLA match | .947 | .533 | 1.683 | .8536 |
| GC dose (2 mg/kg versus <2 mg/kg) | 1.254 | .642 | 2.447 | .5075 |
| Response to GC therapy | .746 | .424 | 1.313 | .3100 |
| Disease risk index | 1.600 | .949 | 2.700 | .0778 |
| Donor type (sibling) | .567 | .292 | 1.100 | .0933 |
| BMI | 1.027 | .956 | 1.104 | .4661 |

* Statistical significance.

Table 7 Multivariate analysis indicating risk factors for death for all reason

| Covariates | HR | 95% CI | | P Value |
|-----------------------------------|-------|--------|-------|---------|
| | | Lower | Upper | |
| Early hyperglycemia | 3.540 | 1.670 | 7.504 | .0010* |
| Age | 1.024 | .999 | 1.051 | .0600 |
| HLA identity | .629 | .329 | 1.205 | .1600 |
| GC dose (2 mg/kg versus <2 mg/kg) | 1.400 | .609 | 3.218 | .4300 |
| Response to GC therapy | .304 | .139 | .665 | .0029* |
| Disease risk index | .836 | .502 | 1.393 | .4900 |
| Donor type (sibling) | .280 | .127 | .616 | .0016* |
| BMI | .983 | .914 | 1.056 | .6300 |

* Statistical significance.

Table 8 Multivariate analysis indicating risk factors for NRM

2.10.3. Implementation of a score

Assuming that a lack of response to GC therapy during the first week of initiation and hyperglycemia are two main risk factors for adverse prognosis in this cohort, we established a score which integrates these two parameters for further analysis.

This score consisted of each 1 point which patients received for either showing hyperglycemia during the first 3 days after GvHD onset and/or non-response to first line steroid therapy within 7 days(199). This scoring system enabled us to allocate patients to three different groups. Patients with normoglycemia and response to GC (n=25) received 0 points, patients with one of these conditions (n=47) received 1 point and patients meeting both criteria received 2 points (n=32). Taking this score into account, patients faced a substantial inferior prognosis with an increasing score. Patients with a score of 0 had a 5 year OS of 75.4%. Patients with a score of 1 and 2 had a HR of 1.42 (p=0.0062) and 5.45 (p=0.002) for mortality compared to patients with a score of 0 (Figure 10). Expressiveness of this score got even more pronounced regarding NRM, showing a 14.3-fold (p=0.011) and 32.9-fold (p=0.0008) higher risk for NRM in patients with a score of 1 and 2, respectively, in comparison to patients with none of these risk factors (Figure 11). However, also here, we did not observe statistical significance showing a predictive risk of relapse (p=0.18) (Figure 12).

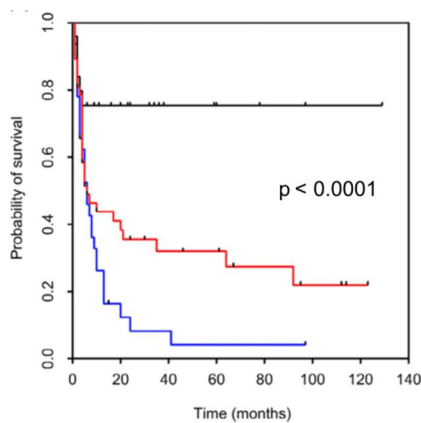


Figure 10 Incidence of survival according to our score (score 0: black line, score 1: red line, score 2: blue line). Increasing score correlated directly to mortality (p<0.0001 for trend). Reproduced from (22) with permission from the copyright holder.

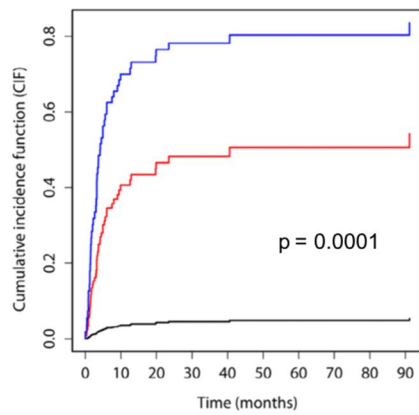


Figure 11 Impact of the score 0: black line, score 1: red line, score 2: blue line) related to a higher NRM indicated by risk analysis on competing causes of death ($p < 0.0001$ for trend). Reproduced from (22) with permission from the copyright holder.

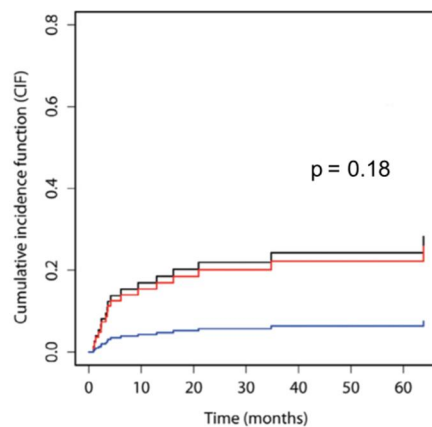


Figure 12 No association of the score (score 0: black line, score 1: red line, score 2: blue line) regarding relapse of underlying disease. The p-values indicate significance for trend derived by log-rank testing. Reproduced from (22) with permission from the copyright holder.

2.11. Discussion

GvHD is a complex and severe disease contributing significantly to non-relapse mortality after allogeneic HSCT. This circumstance is a reason for a limited use of stem cell transplantation which is a potentially curative modality for patients suffering from different malignant and nonmalignant diseases. Clinical grading tools as well as biomarkers are necessary for stratifying and identifying risk for GvHD related complications. In this retrospective analysis we were able to identify hyperglycemia to be a prominent and predictive factor for poor outcome in patients with acute GvHD. Our generated score which includes glycemic status and response to first-line glucocorticoid therapy was highly predictive for mortality and non-relapse mortality in patients with acute GvHD.

Unfortunately less than a half of patients respond to standard steroid therapy and require additional agents for the treatment of GvHD(200, 201). During the last years intensified research on biomarkers and generation of scores has been done in order to improve the available therapy options. MacMillan et al developed a score based on the numbers of organs affected by GvHD and the GvHD severity. In context to this score mortality and transplantation associated mortality were 2 and 2.5- fold higher, respectively in patients with higher scores(202).

Furthermore several biomarkers have been identified to either predict response to steroids or severity and risk of GvHD occurrence(203-208). Multiple correlations were demonstrated using individual and combined markers on outcome, however, these results were mainly inconsistent among different research centers and thus doubtful. Therefore well designed, prospective clinical trials are required to reliably quantify the value of these score and biomarkers, including early hyperglycemia in clinical practice(209).

Hyperglycemia in general is known to be a specific biomarker in several patient populations with or without preexisting diabetes mellitus. Also steroid-induced hyperglycemia was proven to have an influence on patients outcome. Hammer et al. demonstrated a clear correlation between hyperglycemia and adverse outcome during the first 100 days after stem cell transplantation(20). Pidala et al partly refined this finding, by showing that an average glucose above 150 mg/dl and maximum glucose level above 200 mg/dl were related to a reduced overall survival and an increased risk for non-relapse mortality(210). Our results fit well with these findings, moreover we were able to extend these findings by demonstrating that especially early hyperglycemia which appears during the first three days after GvHD onset contributes to a poor outcome. Accordingly, glycemia in the first three days after GvHD onset reflected overall glucose which was collected during the whole period of systemic GC therapy. This result shows that insulin resistance may be present from the onset in patients with adverse-risk GvHD because of high inflammatory burden and it may be exacerbated during start of steroid therapy. Considering this hypothesis, several biomarkers presenting activation of immune system and inflammation have been discovered to identify patients at higher risk of poorer outcome early during the course of GvHD(204, 205, 207).

Despite all these interesting data we collected, it remains still unclear whether hyperglycemia is a non-modifiable marker reflecting general severity of disease or whether intensified treatment of hyperglycemia might also lead to an improvement of outcome in this cohort. Therefore, well designed, randomized controlled trials will be necessary to

answer this crucial question. Our data presented here, might have several implications for these clinical trials in patients with GvHD. Firstly, intensive glucose monitoring needs to be prospectively tested starting by the first day of GC therapy. Secondly, as the risk to die was significantly higher in patients with early hyperglycemia compared to normoglycemic patients, it might be interesting to know whether an early switch to second-line therapy in order to save steroid doses and improve glycemia may be beneficial in patients presenting with early hyperglycemia. Inclusion of our implemented score might have a contributing value in prospective studies. Furthermore, interventions testing novel generations of second-line-therapy for GvHD should include also testing for alterations in glucose to investigate whether alternative therapies which do not influence glucose metabolism that extensively might be more preferable than GC in the treatment of GvHD in future. In conclusion our data demonstrate that monitoring for glucose as an easy and cheap method should be implemented after start of GC therapy in this cohort.

Declaredly our study has some limitations that we have to note. First, it was a monocentric analysis including patients within in- and outpatient hospital settings which represents a heterogeneity of data. Next, we have to mention that this was a retrospective analysis and documentation of other influencing factors as information about duration and dose of parenteral nutrition and detailed data on antihyperglycemic therapy cannot reliably be determined afterwards(22).

2.12. Conclusion

In our retrospective analysis we identified early hyperglycemia after initiation of GC therapy as a potent predictor for poor outcome in patients with acute GvHD. A score based on glycemic state and response to GC was highly predictive for overall survival and non-relapse-mortality. Considering the importance to identify GvHD patients at high risk for adverse outcome we recommend a tight assessment of glucose parameters immediately after diagnosis of acute GvHD incorporated in clinical practice. Next, we believe that prospective trials should be performed to evaluate, whether a tight glycemic control in this population can be achieved and if yes, if it also contributes to a better prognosis(22).

2.13. Publication and presentations in scope of this study

Results from this study were published in March 2017 as original article in “Biology of Blood and Marrow Transplantation” which is a category 1 paper in the field of hematology. The current impact factor for this journal is 3.98.

Furthermore data from this cohort was presented as oral presentation on the 77th scientific session of the American Diabetes Association in June 2017 in San Diego (USA), as poster on the 76th Scientific session of the American Diabetes Association in June 2016 in New Orleans (USA), at the 4th Inhospital Diabetes Meeting in May 2017 (Atlanta, USA) and at the 43rd Jahrestagung der Österreichischen Diabetesgesellschaft in November 2015 in Salzburg, Austria.

3. HYPERGLYCEMIA WITHIN THE FIRST MONTH AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS AN INDEPENDENT RISK FACTOR FOR OVERALL SURVIVAL IN PATIENTS WITH ACUTE MYELOID HYPERGLYCEMIA(211)

Diabetes Metab. 2017 Mar 6. pii: S1262-3636(17)30029-0. doi: 10.1016/j.diabet.2017.02.001. [Epub ahead of print]

Hyperglycaemia within the first month after allogeneic haematopoietic stem-cell transplantation is an independent risk factor for overall survival in patients with acute myeloid leukaemia.

Aberer F¹, Kremser S², Mader JK¹, Zinke-Cerwenka W², Greinix H², Tripolt NJ¹, Pieber TR³, Zebisch A², Sill H², Oulhaj A⁴, Sourij H⁵, Wölfler A⁶.

3.1. Introduction

3.1.1. Definition of acute myeloid leukemia

Acute myeloid leukemia (AML) is a biologically heterogeneous malignant disease arising from myeloid progenitor cells(212). Without treatment AML is limiting life expectancy substantially by related complications including infection, hemorrhage and organ failure(213). Untreated, half of patients with AML die within 5 months after the appearance of first related symptoms and 100 percent showed a lethal outcome after 1 year of disease onset(214). Improvement of diagnostic options and availability of chemotherapeutic therapy could improve outcome by achieving complete remission and prolonging complication free intervals in the 80s(52, 212, 215, 216). During progress in therapeutic options in the next decades mean remission rates in patients younger than 60 years of age could be raised to almost 72% and long lasting remission for 4 to 5 years to 34%(217). However while outcome improved substantially in younger patients in recent years, prognosis of AML in the elder population is still poor(218).

3.1.2. Epidemiology of AML

The average incidence of AML is 3.7/100.000/year and increases with age, showing incidences up 60/100.000 in patients who are older than 70 years. Median age at onset in a Swedish registry was 72 years (219). Males are affected more often (5:3)(220).

3.1.3. Risk factors for AML

Main known risk factors for AML are exposition to nuclear radiation, benzols, tobacco, mineral oil products, herbicides and pesticides. Furthermore several cytostatic acting agents are potentially triggering occurrence of AML by causing aberration in different chromosomes(221). In a meta-analysis of 23 epidemiological studies it was shown that risk of AML in active smokers is 40% and in former smokers 25% higher in contrast to non-smokers(222). Further risk factors are increasing age, male sex, previous cancer treatment and genetic disorders as down-syndrome(221). There is no established and effective preventive measure or early detection possibility except avoiding exposition to risk factors.

3.1.4. Clinical presentation and symptoms of AML

Presence of symptoms or development of AML-related complications is based on increased hematopoietic deficiency induced by bone marrow infiltration of myeloid blasts. Initial symptoms are unspecific and are in the early stages based on concurrent anemia (fatigue, paleness, vertigo), neutropenia (susceptibility to (especially bacterial) infections in different organ systems), and thrombocytopenia (petechial bleedings, ecchymosis, spontaneous hematoma, epistaxis). Moreover, an increased predisposition to bleeding is also induced by a possible disseminated intravascular coagulation and hyperfibrinolysis. The majority of patients presents with leukocytosis and with blasts in the peripheral blood. If leukocytosis increases about over 100.000/ μ l, danger of leukostasis appears, which can lead to acute life- threatening impairments of blood circulation in the lungs or the brain. This circumstance is a hematological emergency and requires urgent reduction of leucocyte amount by chemotherapy, leukapheresis or low-dose radiation(223).

3.1.5. Pathophysiology of AML

Physiologically myeloblasts are immature progenitor cells of the white blood cells produced by the bone marrow. In AML, genetic changes accumulate which inhibit cell differentiation and induce proliferation.. Thus, regulation of cell proliferation, differentiation, survival, self-renewal and repair of DNA are disturbed which causes the clinical entity of AML mostly initially presenting with pancytopenia and it's clinical manifestations(224). Secondary AML arises based on a different underlying clonal

disorder of hematopoiesis mostly from myelodysplastic syndrome, chronic myeloproliferative neoplasia or after exposure to a leukemogenic agent(225).

3.1.6. Diagnosis of AML

AML is a complex and progressive disease characterized by multiple somatically acquired mutations, coexisting molecular clones and disease evolution of time. New diagnostic approaches of gene sequencing techniques were able to identify 23 genetic mutations which commonly occur in AML patients. The classification of AML is based on disease morphology, (molecular) cytogenetics, immunophenotype and clinical features(226). At the time of diagnosis, cytogenetic and molecular analyses allow stratifying of patient's risk as favorable, intermediate or adverse in context to disease outcome(227).

The tentative diagnosis arises from clinical symptoms and blood counts.

Primary diagnosis is often made accidentally by identifying pancytopenia. Diagnosis needs to be confirmed by bone marrow biopsy. The current diagnostic approach is based on molecular and cytochemical investigations, supplemented by immunophenotyping, and cytogenetics.

An AML diagnosis includes:

- On blood smears at least 200 leukocytes and on spiculated marrow smears 500 nucleated cells should be counted. A count of more than 20% of blasts (myeloblasts, monoblasts and megakaryoblasts) is required for the diagnosis.
- Classification of blasts to the myeloid lineage by cytochemical investigations and immunophenotyping
- Further classification to a subtype appropriate to the FAB- and WHO classification

| Objective | Investigation |
|--|--|
| Diagnosis confirmation | Clinical history and medical status |
| | Blood count and differential count |
| | Bone marrow biopsy and cytochemistry |
| | Immunophenotyping |
| | Cytogenetic analyses |
| | Gene mutations |
| | <ul style="list-style-type: none"> ● NPM1 ● CEBPA ● RUNX1 ● FLT3 ● TKD (Codon D853 und I836) ● TP53 ● ASXL1 |
| | Gene rearrangements |
| | <ul style="list-style-type: none"> ● PML-RARA ● CBFβ-MYH11 ● RUNX1-RUNX1T1 |
| | Additional tests and procedures at diagnosis |
| Analyses of comorbidities (e.g. HCT-CI Score) | |
| Demographics, family and medical history | |
| Biochemistry, coagulation tests urine analysis | |
| Pregnancy test | |
| Fertility conserving procedures (oocyte or sperm cryoconservation) if applicable | |
| HLA- Typing (if applicable also siblings) + CMV Status (for patients who face stem cell transplantation) | |
| Hepatitis A, B, C - and HIV testing | |
| Chest radiography, electrocardiogram, echocardiography, pulmonary function testing | |

Table 9 Diagnostic procedures for AML according to (226)

3.1.7. Classification of AML

Two systems for classification of AML are used. The old French-American-British (FAB) classification, which is based on morphology to define specific subtypes, and the classification of the world health organization (WHO) which focuses on chromosomal translocations, biological and clinical features and evidence of dysplasia(228, 229).

| Subtype | Specification |
|--|---|
| Acute Myeloid Leukemia with recurrent genetic aberrations | AML with t(8;21)(q22;q22); RUNX1-RUNX1T1 |
| | AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 |
| | APL with t(15;17)(q22;q12); PML-RARA |
| | AML with t(9;11)(p22;q23); MLLT3-KMT2A |
| | AML with t(6;9)(p23;q34); DEK-NUP214 |
| | AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); GATA2, MECOM |
| | AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1 |
| | Provisional entity: AML with BCR-ABL1 |
| | AML with mutated NPM1 |
| | AML with biallelic mutations of CEBPA |
| Provisional entity: AML with mutated RUNX1 | |
| Acute myeloid leukemia with myelodysplasia-related | |
| Therapy-related myeloid neoplasms | |
| Acute myeloid leukemia, not otherwise specified (NOS) | Acute myeloid leukemia with minimal differentiation |
| | Acute myeloid leukemia without maturation |
| | Acute myeloid leukemia with maturation |
| | Acute myelomonocytic leukemia |
| | Acute monoblastic/monocytic leukemia |
| | Pure erythroid leukemia |
| | Erythroleukemia, erythroid/myeloid |
| | Acute megakaryoblastic leukemia |
| | Acute basophilic leukemia |
| | Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute |
| Myeloid sarcoma | |
| Myeloid proliferations related to Down-syndrome | Myeloid leukemia associated with Down syndrome |
| | Transient abnormal myelopoiesis (syn.: transient myeloproliferative disorder) |
| Acute leukemia of ambiguous lineage | Acute undifferentiated leukemia |
| | Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); BCR-ABL1 |
| | Mixed phenotype acute leukemia with t(v;11q23); MLL |
| | Mixed phenotype acute leukemia, B/myeloid, NOS |
| | Mixed phenotype acute leukemia, T/myeloid, NOS |

Table 10 WHO classification for AML according to (226)

| Subtype | | Percentage in adults |
|---------|---|----------------------|
| M0 | Undifferentiated acute myeloblastic leukemia | 5 |
| M1 | Acute myeloblastic leukemia with minimal maturation | 15 |
| M2 | Acute myeloblastic leukemia with minimal maturation | 25 |
| M3 | Acute promyelocytic leukemia (APL) | 10 |
| M4 | Acute myelomonocytic leukemia | 20 |
| M4eos | Acute myelomonocytic leukemia with eosinophilia | 5 |
| M5 | Acute monocytic leukemia | 10 |
| M6 | Acute erythroid | 5 |
| M7 | Acute megakaryoblastic leukemia | 5 |

Table 11 The French-American-British (FAB) classification for AML according to (229)

3.1.8. Prognostic factors for AML

Risk stratification is an important tool in modern medicine in order to decide about therapeutic approaches. Several patient-related and leukemia-related risk factors were identified. However we were able to supplement already existing risk factors by glucose (see chapter 3.2).

3.1.8.1. Age

Age at AML diagnosis is the most potent patient-related risk factor influencing prognosis(230). In the elderly many factors contribute to an impaired prognosis: general performance status, contraindications against chemotherapeutic treatment, intercurrent comorbidities, a higher incidence of secondary AML and a higher risk for adverse cytogenetic abnormalities(231).

3.1.8.2. Performance status and geriatric conditions

Patients with a reduced performance status as assessed by ECOG status showed an adverse prognosis independent of age, and especially the combination of higher age and impaired performance status is highly predictive for early death after treatment induction(232). A deficit in the activities of daily living assessed by the Barthel Index was also an independent risk factor for overall survival in patients with AML(233).

3.1.8.3. Cytogenetic risk

The cytogenetic risk is the most prominent leukemia-related risk factor for outcome in patients with AML after chemotherapy. Patients with balanced translocations with involvement of the core binding protein transcription factor complex have a more favorable risk than patients with complex cytogenetic aberrations(234). In numbers a better-risk disease has a long-term survival of more than 65%, a medium-risk disease is connected to a survival of 25% and patients with cytogenetically poor-risk disease face a long-term survival lower than 10%(235).

3.1.8.4. Gene mutations

Distinct gene mutations have also been identified to contribute to an adverse risk in AML patients. For instance, mutations in the FLT3 gene resulting in an internal tandem duplication (FLT3-ITD) have a poor outcome, while patients with a sole aberration in the NPM1 gene or patients with biallelic CEBPA gene mutation have a relatively good prognosis. New diagnostic approaches like targeted parallel DNA sequencing might increase the number of informative gene mutations concerning prognosis in the future(230).

3.1.8.5. Common laboratory parameters

A high peripheral leukocyte count and increased lactat dehydrogenase (LDH) levels were connected to an adverse prognosis in AML patients(230, 236). Furthermore hemoglobin value, platelet count and fibrinogen plasma level were associated to a poor prognosis in several outcome measures(236, 237).

3.1.2. Treatment of AML

Therapeutic measures in patients with AML are dependent on patient's age and constitution.

Therapeutic option of choice in most patients with AML is intensive chemotherapy which can be categorized into three phases.

3.1.2.1. Induction therapy

Induction therapy should be initiated as soon as possible after AML diagnosis aiming to restore normal bone marrow function. Even a delay of 5 days was associated with an adverse effect on outcome(238).

Standard-induction therapy contains the combination of application of an anthracycline (e.g. daunorubicin, idarubicin or mitoxantron) for 3 days and a 7-day cyclus of cytarabine. Aim of induction therapy is to clear the bone marrow of malignant cells and to achieve disease remission, which is defined by less than 5% blasts in the bone marrow. Remission can be observed after the first cycle in most patients. About a quarter of patients require a second induction cycle to obtain complete remission status. Patients who do not respond to first line induction therapy combination will be treated with salvage chemotherapy. Induction therapy is highly toxic mainly because of the effect on hematopoiesis, which can provoke infectious or bleeding disorders(239).

3.1.2.2. Post-remission-therapy

Patients who achieved complete remission (CR) require immediate consolidation therapy to maintain remission status and to prevent relapse of AML. Post-remission-therapy includes application of cytarabine or allogeneic stem cell transplantation. Choice of these therapeutic options is based on the risk profile of the AML and the patient's constitution(239).

3.1.2.3. Allogeneic hematopoietic stem cell transplantation (HSCT)

HSCT is a highly complex and intensive therapy which offers high curative potential but also contributes to a high patient risk for related morbidity and mortality. HSCT is performed in specialized and certified institutions which offer appropriate diagnostic, personal, regulatory and sanitary conditions. The aim of performing HSCT is on the one

hand to replace the disease-affected hematopoietic system of the patient but also on the other hand to reconstitute the patient with a new and fully functional immune- and hematopoietic system, which is able to attack residual leukemic cells.

HSCT is indicated when physicians expect a superior prognosis indicating that disease related morbidity and mortality is improvable by conducting HSCT(240). Kind and probability of HSCT- related complications are highly connected to compatibility of donor tissue and stem cell origin(241). Main indications for HSCT are all kinds of acute and chronic leukemia, lymphoma and myelodysplastic syndromes(242). In severe cases of benign disorders of hematological disease as thalassemia, sickle cell anemia, aplastic anemia or special autoimmune diseases HSCT can also be considered(243).

Before allogeneic hematopoietic stem cells are infused, the patient need to receive chemotherapy with or without concomitant radiotherapy. The used conditioning regimens can be divided according to their intensity (myeloablative or non-myeloablative). Decisive factors, which influence the intensity of conditioning are comorbidity, age, remission status prior to transplantation and risk of relapse.

Aim of the conditioning therapy is

- induction of host immunosuppression to allow engraftment and to minimize the risk of graft rejection
- providing antileukemic potency in order to eliminate as much malignant cells as possible
- induction of myeloablation in order create space for transplanted donor cells

Standard conditioning regimens include full-body radiation plus application of cyclophosphamide or busulfan plus cyclophosphamide(228, 244, 245).

After conditioning, donor hematopoietic stem cells are infused intravenously, which then homeautonomously to the bone marrow. Usually regeneration of hematopoiesis (engraftment) is seen within two to three weeks after transplantation.

Genotypical investigations (chimerism analysis) after transplantation are necessary in order to evaluate whether the regenerated hematopoiesis has its origin from the donor or the recipient, which is indicative of residual leukemic cells. The main aim is to achieve and

maintain complete donor chimerism, which means that bone marrow function is completely based on transplanted donor cells with no evidence of residual leukemic cells.

Complications of HSCT are direct toxic effects of chemotherapeutics or radiation as inflammation of mucosal tissue, nausea, emesis, cystitis or other organ specific side effects. Additional complications include the development of acute or chronic Graft-versus-Host disease (further description in an upcoming chapter), development of secondary malignoma and infection as well as bleeding complications. Despite the fact that HSCT is a potentially curative therapeutic option, the risk of relapse of the underlying disease is not definitely banned(246).

3.1.3. Previously conducted trials focusing on hyperglycemia in AML patients.

A retrospective cohort study, which was performed in the United States (2002-2005), included 283 patients with AML. Patients were followed for 3 years after AML diagnosis and glucose parameters were collected during this time. Authors observed a clear correlation of hyperglycemia and hospital mortality (HR 1.38; $p < 0.01$) after adjusting for several covariates. Even mild hyperglycemia led to a significantly higher mortality. Furthermore hyperglycemia was also correlated to a higher risk of sepsis and respiratory failure. A correlative calculation of risk of hyperglycemia and GvHD was unfortunately not determined in this analysis. Furthermore steroid therapies and doses were not taken into account(19).

Gebremedhin et al. investigated 328 patients, who underwent HSCT for acute leukemia. They observed that hyperglycemia which appears immediately after allogeneic HSCT indicated an increased likelihood of acute GvHD, but interestingly only in normal-weight study participants(21).

Hammer and coauthors published results of a retrospective trial which included 1.175 HSCT recipients between 2000 and 2005. They focused not only on hyper- but also the hypoglycemic state after HSCT. The HR for death was 1.93 for patients with arbitrary glucose values >200 and even 2.78 for patients which showed values >300 mg/dl. A minimum glucose <89 mg/dl was associated with a 200 days non-relapse-mortality OR of 2.17, indicating that not only hyper- but also hypoglycemia increases risk of adverse outcome in this patient cohort(20).

3.2. Aim of this study

The aim of this study was to retrospectively investigate the effect of hyperglycemia during one month after HSCT on survival rates in patients who underwent HSCT for AML(211).

3.3. Study design, parameters of interest, regulatory issues

This study was a monocentric, retrospective data analysis including 159 AML patients which were treated with HSCT at the Division of Hematology at the Medical University of Graz during the years 1996 and 2013. Data was thankfully collected by Dr. Sonja Kremser who performed her diploma thesis under the supervision of Prof. Albert Wölfler. The scope of her diploma thesis was the evaluation of general outcome parameters and identification of risk parameters of patients who underwent HSCT for AML. Data were obtained by individual medical records and the medical documentation and communication system (MEDOCS). Data and parameters of interest which were investigated were age at AML diagnosis, type of AML, gender, blood count and lactatdehydrogenase (LDH), cytogenetic and molecular aberrations at diagnosis, date of HSCT, disease remission status at HSCT, type of HSCT including stem cell source, human leukocyte antigen (HLA)-compatibility, steroid therapy, CMV status of donor and recipient, comorbidities, presence (and if date) of relapse, date of death or last contact, overall survival and especially important for this study, blood glucose values which were collected during the first month after HSCT. The study was approved by the local Ethics committee (registration number EK27-082ex14/15) and conducted in full accordance to the declaration of Helsinki and good clinical practice (GCP) requirements. By the fact that it was a retrospective, non-interventional investigation, no informed consent was needed.

3.4. Statistical analysis

Anonymized patient data were documented in EXCEL©2010. Statistical analyses were performed using R version software (R Foundation of Statistical Computing, Vienna, Austria).

The main variable of interest in this study was the time duration (in months) from HSCT until death. The main exposure variable was glycemie status during 1 month after HSCT.

Kaplan-Meier survival plots were used to calculate survival curves related to the main event (death), according to glycemic status. Cox-proportional hazards models were used to determine the factors which influence death after adjusting for other covariates (age at diagnosis, sex, HLA- compatibility, stem-cell source, cytogenetic AML risk score and steroid use). A p-value <0.05 was deemed to indicate statistical significance.

3.5. Material and methods

Glucose data were collected during the first twenty-eight days after HSCT for 159 AML patients. By the fact that it was not possible to precisely insure that collected values were all pre-prandial values in retrospect, we pragmatically divided study participants in four glucose categories (GC). GC1 contained patients who showed no single glucose value higher than 125 mg/dl during hospital stay. GC2 included patients with less than a quarter of all glucose values higher than 125 mg/dl. Patients who were allocated to GC3 had more than 25% of their glucose values higher than 125 mg/dl but without passing a threshold >250 mg/dl. Patients in GC4 had at least one glucose value of higher than 250 mg/dl. Distribution of participants in glucose categories 1-4 was 37, 62, 38 and 22. In the analysis we compared each category against each other(211).

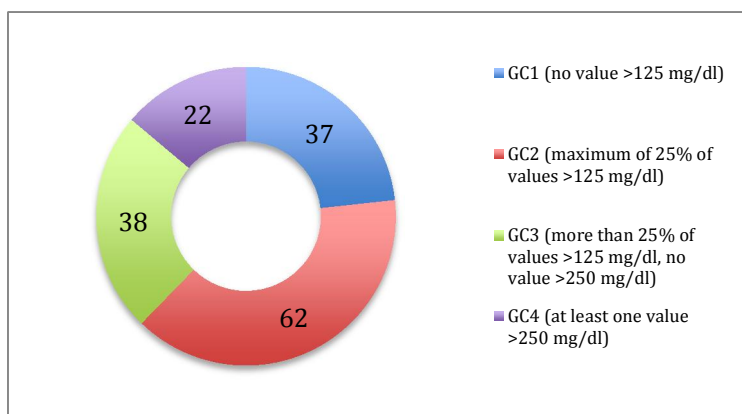


Figure 13 Distribution of patients in different glucose categories

3.6. Results

We performed a retrospective analysis in 159 patients who received allogeneic HSCT for the treatment of acute myeloid leukemia. 45% of the participants were female, mean age of the cohort was 50 ± 13 years. A detailed table of baseline characteristics is outlined below.

| | | |
|------------------------|----------------------------|-----------|
| Gender | female | 72 (45%) |
| | male | 87 (55%) |
| Cytogenetic risk (ELN) | favourable | 5 (3%) |
| | intermediate-1 | 73 (46%) |
| | intermediate-2 | 40 (25%) |
| | poor | 29 (18%) |
| | not available | 12 (8%) |
| Stem cell source | peripheral blood | 141 (89%) |
| | umbilical cord blood | 18 (11%) |
| | bone marrow | 0 (0%) |
| HLA compatibility | matched donor | 113 (71%) |
| | mismatched donor | 46 (29%) |
| Steroid therapy | yes | 23 (14%) |
| | no | 136 (86%) |
| Disease status at HSCT | complete remission 1 (CR1) | 99 (62%) |
| | not in CR1 | 60 (38%) |
| Conditioning therapy | myeloablative | 42 (26%) |
| | non-myeloablative | 117 (74%) |
| Cause of death (n=76) | relapse | 40 (53%) |
| | non-relapse | 36 (47%) |

Table 12 Baseline characteristics

A preexisting diabetes was known in 6 of the patients (4%). 33 subjects (21 %) of the patients required systemic (oral or intravenous) steroid therapy during the hospital stay. In total 76 patients (47%) died during time of observation, the majority within the first year after HSCT.

The median follow-up duration was 31.8 months (interquartile range (IQR): 11.7-65.6) Patients who were allocated to glucose category 1 (GC1) had a median follow up of 31.8 months (IQR 13.8-85.5) patients in the glucose categories 2,3 and 4 presented with a median follow up of 31.6 (IQR: 9.6-57.7), 34.8 (IQR: 18.6-54.4) and 24.6 (IQR: 13.1-72.1) months. Kaplan Meier survival curve is displayed below.

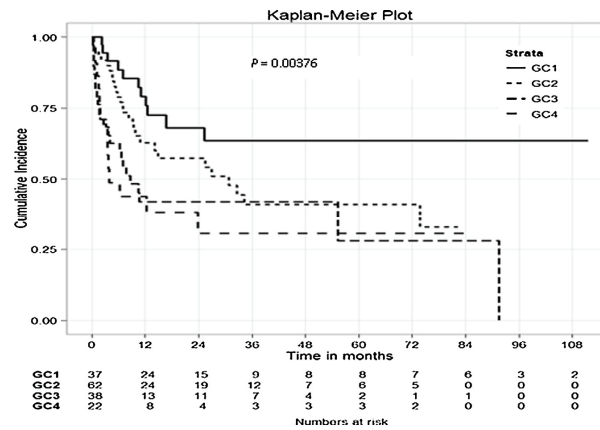


Figure 14 Kaplan Meier survival curves indicating cumulative incidence of death as outcome according to the defined glucose category (GC) within the first month after HSCT for AML. The unadjusted p-value is derived by log-rank tests and rejects the null hypothesis that the survival curves for all four patients are similar. Reproduced from (211) with permission from the copyright holder.

Subjects of categories 2,3 and 4, those who had at least one glucose value >125 mg/dl during the month of observation, faced a 2.5- fold increased risk of mortality in comparison to those with no elevated glucose reading. Overall mortality rates in patients which were allocated to GC 3 and 4 were significantly higher as in GC1, even after adjustment for age at diagnosis, gender, HLA- compatibility, stem cell source and steroid use (HR 2.81 (95%-CI 1.31-6.03) for GC3 and HR 2.75 (95%-CI 1.21-6.25)) for GC4. The HR for mortality in GC2 was 1.56 (95%-CI 0.75-3.24) and did not show statistical significance. However, no significant differences in mortality rates were demonstrated between GC2 and GC3 (p=0.058), GC2 and GC4 (p=0.091) or GC3 and GC4 (p=0.955)(211).

| a.) Unadjusted univariate analysis | | |
|---|--------------------|-----------------|
| <i>Variable</i> | <i>HR (95%-CI)</i> | <i>P- Value</i> |
| GC1 | na | na |
| GC2 | 1.95 (0.96, 3.95) | 0.062 |
| GC3 | 3.02 (1.46, 6.22) | 0.003 |
| GC4 | 3.54 (1.59, 7.85) | 0.002 |
| b) Adjusted multivariate analysis | | |
| <i>Variable</i> | <i>HR (95%-CI)</i> | <i>P- Value</i> |
| GC1 | na | na |
| GC2 | 1.56 (0.75 , 3.24) | 0.232 |
| GC3 | 2.81 (1.31 , 6.03) | 0.008 |
| GC4 | 2.75 (1.21 , 6.25) | 0.015 |
| Age at diagnosis | 0.99 (0.97 , 1.01) | 0.296 |
| Gender | 1.16 (0.72 , 1.85) | 0.542 |
| HLA-compatibility | 0.73 (0.39 , 1.36) | 0.320 |
| Stem cell source | 1.64 (0.77 , 3.50) | 0.204 |
| Cytogenetic risk | 1.06 (0.88 , 1.29) | 0.530 |
| Steroid therapy | 1.10 (0.62 , 1.95) | 0.734 |

Table 13 Variables impacting mortality in cox proportional hazard analysis model (CoxPH)

3.7. Discussion

Data obtained by this retrospective analysis demonstrated a significant association between hyperglycemia occurring within the first month after HSCT and overall mortality in patients with AML.

Hammer et al.(20) published that malglycemia (including also hypoglycemia) is a substantial indicator for non-relapse mortality in patients who underwent HSCT. However this publication included patients with all kinds of hematological malignancies, while we took our focus on patients with AML only. It is well known that hyperglycemia, with or without previously identified diabetic disease, contributes adversely to outcome in various patients populations and settings (247) and we were able to confirm this finding in our study. This potent predictor for adverse outcome might present an essential element in future risk scores which are established in modern medicine.

A limitation of this study is its retrospective nature and lack of glucose profiles during the hospital stay. Nevertheless this limitation does not significantly weaken our overall conclusion that any glucose reading >125 mg/dl during the hospital stay is related to adverse prognosis. However, it is still not possible to distinguish whether hyperglycemia presents a predictor of mortality or might be a modifiable biomarker. To answer this question we need large and prospective, randomized controlled trials.

In conclusion this study revealed a potential impact of hyperglycemia on outcome in patients with AML after HSCT. Therefore we postulate and recommend glucose profiling, as easy available and cheap method, regularly in this cohort as it represents a clear predictor of negative outcome(211).

3.8. Conclusion

Our findings identified high glucose to be a substantial predictor for mortality in AML patients who underwent HSCT(211).

3.9. Publication and presentations in scope of this study

Results from this trial were published in February 2017 as research letter in “Diabetes and Metabolism” which is a category 1 paper in the field of endocrinology and diabetology. The current impact factor for this journal is 4.7.

Furthermore data were presented as poster on the 76th Scientific session of the American Diabetes Association in June 2016 in New Orleans (USA) and at the 43rd Jahrestagung der Österreichischen Diabetesgesellschaft in November 2015 in Salzburg, Austria.

4. A PILOT TRIAL TO INVESTIGATE FEASIBILITY AND SAFETY OF AN AUTOMATIZED DECISION SUPPORT SYSTEM (GLUCOTAB®) FOR THE TREATMENT OF STEROID-INDUCED HYPERGLYCEMIA IN SUBJECTS WITH ACUTE GRAFT-VERSUS-HOST-DISEASE

4.1. Introduction

High glucose levels, appearing with or without previously known diabetes mellitus, are a common and widespread health care problem with substantial medical consequences. Increasing evidence indicates that hyperglycemia during acute illness is not a physiologic or irrelevant condition which normalizes during recovery. Much more, hyperglycemia in hospitalized patients showed to be a predictive marker of poor clinical outcome and mortality(2). Insulin represents the therapy of choice in patients who are admitted to hospital for acute disease and for glucose adjustment in hospitalized patients insulin titration protocols are recommended by current guidelines(248). The GlucoTab is a decision support system and bedside workflow system with the objective to improve glycaemic control and facilitate daily handling of hyperglycemia in hospitalized patients.

4.1.1. Intended use of the GlucoTab system

The GlucoTab system was invented during the last decade from the Medical University of Graz in collaboration with Joanneum research HEALTH. GlucoTab is a computerized algorithm-based software system developed to support healthcare professionals in the routine treatment of hyperglycemia during hospitalization.

The two main functionalities, GlucoTab offers, are:

- 1) Support of clinical healthcare professionals and nursing staff to manage the treatment of patients with type 2 diabetes mellitus or new onset hyperglycemia by providing automated workflow support, including reminders for open tasks, facilitating documentation and offering visualization of glucose values, nutrition and medication documentation.

- 2) Automated calculation and proposal of total daily insulin dose, prescribed by the physician in charge during the ward round and insulin dosage suggestions for individual insulin administrations premeal and at bedtime.

GlucoTab is a client-server system which is connected to the central hospital server via WIFI. The backend server stores the data and implements the interface to the interface to the hospital information system.

The GlucoTab is licensed to be used in patients with previously diagnosed type 2 diabetes mellitus and newly onset hyperglycemia in hospitalized patients.

GlucoTab use is prohibited for patients with type 1 diabetes mellitus, severe hypoglycemic episodes in the past and gestational diabetes.

4.1.2. Functionality of the GlucoTab system

The automated decision support system is based on a basal/bolus insulin titration protocol (REACTION algorithm). In the hereby described study the long-acting insulin glargine (Lantus®) and the short acting insulin aspart (Novorapid®) were used. The recommendations for insulin therapy consist of a daily dose of basal insulin and short acting insulin for covering meals and correction of hyperglycemia. The proportion of basal and bolus insulin is calculated with 50:50 percent. GlucoTab is aiming to implement a glucose target between 70 and 140 mg/dl premeal. Insulin therapy is initiated with 0.5 insulin units/ kg body weight. In patients older than 70 years old and a serum creatinine level >2 mg/dl initial is reduced to 0.3 IU/kg. If patients were previously on a satisfying insulin therapy regimen, initial dose can be manually adjusted to the original dose.

Previously conducted trials in non critically ill patients with diabetes mellitus type 2 or newly diagnosed hyperglycemia which were hospitalized on internal and surgical wards showed an increase of percentage of glucose values in target and less hypo- and hyperglycemia in comparison to a cohort of patients which were treated with paper based treatments prescribed by physicians in charge. Furthermore GlucoTab was well accepted by medical and nursing staff which led to a high adherence to the suggested therapy proposals(163, 249, 250).



Figure 15 The Gluco Tab System. Pictures reproduced from the GlucoTab investigator brochure with permission of Joanneum research.

Acute Graft-versus-host disease (GvHD) represents a critical and potentially life-threatening complication in patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) for hematological malignancy, occurring in 35-50% of transplant recipients within the first 100 days after HSCT(185). GvHD is characterized by an activation of donor T-cell damage leading to tissue necrosis mostly affecting the skin, gastrointestinal tract or liver(251).

As first-line standard therapy, high-dose systemic glucocorticoids (GCs), mostly administered with 2 mg/kg bodyweight Prednisolone or equivalent, are recommended(252).

One of the miscellaneous complications of steroid therapy is the development of steroid induced hyperglycemia (SIHG) which derives mainly by GC induced increase of hepatic gluconeogenesis and decrease of peripheral insulin sensitivity(98).

A previously published retrospective analysis from our study group demonstrated a clear and substantial negative impact of hyperglycemia on overall survival in acute GvHD patients who were hyperglycemic after initiation of systemic glucocorticoid therapy(253).

However it remains unclear whether hyperglycemia is an independent predictor for adverse outcome or might be a modifiable risk factor.

In general, treatment of SIHG is complex and not standardized by guidelines yet. However, insulin treatment represents the most widely established therapeutic option of choice, especially in severe illness as it is instant in patients with GvHD.

During the last decades, computerized algorithms and workflow/decision support systems for basal-bolus insulin therapy, as the above described GlucoTab, have been developed

which substantially improved glycemic control in non-severely ill hospitalized patients with type 2 diabetes or new onset hyperglycemia(250).

Yet, these algorithms have not been tested for patients with SIHG.

4.2. Aim of the study

The aim of this study was to evaluate the feasibility of such a decision support system, GlucoTab®, in the treatment of GvHD patients with SIHG.

4.3. Study Design, Material and Methods

4.3.1. Study design

We performed a monocentric, randomized controlled pilot trial in 10 hospitalized GvHD patients who developed hyperglycemia after initiation of systemic GC therapy. Hyperglycemia was defined as two fasting values higher than 140 mg/dl.

The study took place either at the transplantation or general ward at the Department of Hematology, Medical University of Graz, Austria.

Randomization was performed with the randomization tool (www.randomizer.at) provided from the institute for Medical Informatics of the Medical University of Graz (randomizer.at). The analysis was approved by the local ethics committee (EK –number 27-116 ex 14/15); all patients gave written consent before initiation of any study procedure.

Patients who were allocated to the intervention group were treated by GlucoTab®, the remaining 5 patients received antihyperglycemic treatment upon the discretion of the medical practitioner in charge. Follow up-duration was 6 months. When patients were readmitted to hospital, they were allocated to the treatment group due to their previous randomization result.

4.3.2. Gluco Tab

This decision support system (DSS), integrated in a mobile, handheld tablet computer, suggests basal-bolus insulin therapy provided by the incorporated standardized insulin dosing algorithm.

Initial insulin therapy starts with 0.5 insulin units/ kg of body weight, if patients are older than 70 years or creatinin passes a threshold of 2.0 mg/dl, initial dose is reduced to 0.3 units/kg of body weight. GlucoTab therapy requires capillary glucose measurements 4 times daily (premeal and bedtime) and proposes either bolus insulin (insulin aspart, NovoNordisk, Bagsvaerd, Denmark) premeal and as correction insulin or basal insulin (insulin glargine, Sanofi-Aventis, Frankfurt am Main, Germany), in a proportion of 50% each, once daily. Therapy proposals can be overruled at any time by medical staff if deemed necessary or reasonable. Further declarations for related features are described elsewhere(250).

In the conventional arm, antihyperglycemic therapy was prescribed by the physician in charge. We requested glucose profiling premeal and bedtime throughout the trial also for patients in the conventional treated group.

4.3.3. Statistical analysis

For collection of baseline and GvHD characteristics we used descriptive statistics. For calculation of mean glucose we performed explorative data analysis. For comparative analysis and significance testing, we conducted paired T-tests, the p-value was derived by the Levene-method; for graphic illustration we used Box plots. All statistic testings were performed with SPSS Version 22.0 (IBM. USA). By the fact that this was a feasibility trial. no sample size calculation was required.

4.4. Results

4.4.1. Patient characteristics

We included 10 patients (female n=7) in this randomized controlled interventional trial. Median age in the GlucoTab arm was 55.2 ± 13.4 vs. 60.2 ± 3.7 years in the conventional group. Only 2 of ten participants (one in each group) were alive after 6 months of observation. Mean duration till death was 80.0 ± 51.6 in the GlucoTab vs. 122.3 ± 52.0 days in the conventional group (exclusive the 2 survivors). Percentage of days being hospitalized during study duration was 45.3 ± 38.1 and 77.8 ± 18.5 for patients who were allocated to the conventional arm. Further patient characteristics are indicated in table 14.

| | GlucoTab (n=5) | ConventionalGroup (n=5) |
|------------------------------|----------------|-------------------------|
| Gender (female) | 3/5 | 4/5 |
| Age (years) | 55.2 ±13.4 | 60.2 ±3.7 |
| BMI | 22.8 ±4.6 | 24.5 ±5.5 |
| Underlying disease | | |
| • AML | 1 | 1 |
| • Secondary AML from MDS | 2 | 3 |
| • Therapy associated AML | 1 | 0 |
| • AML post radiatio | 1 | 0 |
| • ALL | 0 | 1 |
| GvHD onset after HSCT (days) | 27 ±19 | 26.6 ±5 |
| GvHD Location | | |
| • Skin | 3 | 4 |
| • Gastrointestinal Tract | 4 | 5 |
| • Liver | 0 | 1 |
| Overall grading (Glucksberg) | | |
| • 1 | 0 | 0 |
| • 2 | 2 | 3 |
| • 3 | 0 | 0 |
| • 4 | 3 | 2 |
| Donor | | |
| • Relative | 1 | 2 |
| • Unrelated | 4 | 3 |

Table 14 Patient characteristics

4.4.2. Primary outcome parameters

4.4.2.1. Mean glucose

In total 440 vs. 980 glucose values were available in the GlucoTab group and conventional group during systemic GC therapy. Mean overall glucose during time of GC therapy was 165 ± 53 vs. 170 ± 45 mg/dl [$p=0.001$]. Mean morning glucose was 137 ± 38 vs. 152 ± 40 mg/dl [$p<0.001$]. Glucose values at lunchtime, evening and bedtime were comparable with no statistically significant difference. Mean glucose during defined time points are shown in table 15. Figure 16 shows Box plots of mean glucose during different time points.

| | GlucoTab (n=5) | | ConventionalGroup (n=5) | | p |
|------------|----------------|-----|-------------------------|-----|--------|
| | mg/dl | n | mg/dl | n | |
| PG morning | 137.9 ±38.3 | 122 | 152.7 ±40.2 | 277 | <0.001 |
| PG lunch | 155.5 ±47.7 | 108 | 159.2 ±39.7 | 272 | 0.45 |
| PG evening | 191.8 ±64.8 | 110 | 189.7 ±55.6 | 247 | 0.75 |
| PG bedtime | 176.8 ±62.1 | 100 | 178.8 ±47.4 | 184 | 0.72 |
| Mean/Total | 165.5 ±53.2 | 440 | 170.1 ±45.7 | 980 | <0.001 |

Table 15 Mean glucose values during different time points

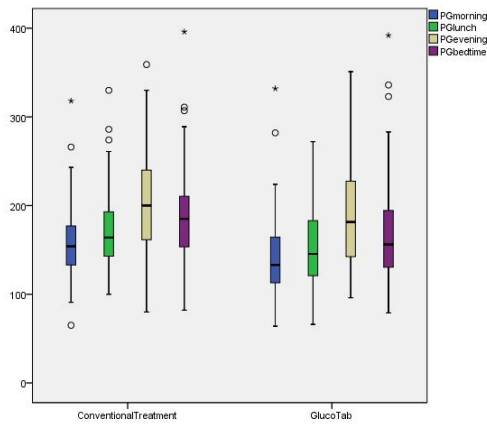


Figure 16 Mean glucose values during different time points

4.4.2.2. Percentage of glucose values in target

68.5% vs. 65.0% of all values were in the recommended target range between 70 and 180 mg/dl. Glucose values above 300 mg/dl appeared slightly more often in the GlucoTab Group (3.8 vs. 1.3%). A more detailed information about distribution of glucose values in target areas is indicated in table 16.

| | GlucoTabGroup (n=5) | | ConventionalGroup (n=5) | |
|--------------------------|---------------------|-----|-------------------------|-----|
| | % | n | % | n |
| PG morning <70 mg/dl | 1.6 | 2 | 0.7 | 2 |
| PG morning 70-110 mg/dl | 19.7 | 24 | 11.2 | 31 |
| PG morning 110-180 mg | 65.6 | 80 | 65.3 | 181 |
| PG morning 180-300 mg/dl | 12.3 | 15 | 22.0 | 61 |
| PG morning >300 mg/dl | 0.8 | 1 | 0.7 | 2 |
| Total morning | 100 | 122 | 100 | 277 |
| PG lunch <70 mg/dl | 0.9 | 1 | 0.4 | 1 |
| PG lunch 70-110 mg/dl | 17.6 | 19 | 3.3 | 9 |
| PG lunch 110-180 mg | 53.7 | 58 | 72.4 | 197 |
| PG lunch 180-300 mg/dl | 27.8 | 30 | 23.2 | 63 |
| PG lunch >300 mg/dl | 0 | 0 | 0.7 | 2 |
| Total lunch | 100 | 108 | 100 | 272 |
| PG evening <70 mg/dl | 0 | 0 | 0.8 | 2 |
| PG evening 70-110 mg/dl | 5.5 | 6 | 4.5 | 11 |
| PG evening 110-180 mg | 44.5 | 49 | 44.1 | 109 |
| PG evening 180-300 mg/dl | 40.9 | 45 | 48.2 | 119 |
| PG evening >300 mg/dl | 9.1 | 10 | 2.4 | 6 |
| Total evening | 100 | 110 | 100 | 247 |
| PG bedtime <70 mg/dl | 0 | 0 | 0 | 0 |
| PG bedtime 70-110 mg/dl | 8 | 8 | 6 | 11 |
| PG bedtime 110-180 mg | 57 | 57 | 44.1 | 88 |
| PG bedtime 180-300 mg/dl | 29 | 29 | 48.2 | 82 |
| PG bedtime >300 mg/dl | 6 | 6 | 2.4 | 3 |
| Total bedtime | 100 | 100 | 100 | 184 |
| PG overall <70 mg/dl | 0.7 | 3 | 0.5 | 5 |
| PG overall 70-110 mg/dl | 13.0 | 57 | 6.3 | 62 |
| PG overall 110-180 mg | 55.5 | 244 | 58.7 | 575 |
| PG overall 180-300 mg/dl | 27.0 | 119 | 33.1 | 325 |
| PG overall >300 mg/dl | 3.8 | 17 | 1.3 | 13 |
| Total | 100 | 440 | 100 | 980 |

Table 16 Distribution of glucose values in different target areas

4.4.2.3. Hypoglycemia

Hypoglycemia defined as glucose value below 70 mg/dl appeared rarely in both groups (0.7 vs. 0.5%). None of the hypoglycemic events required third party help or contributed to a critical situation.

4.5.3. Secondary outcome parameters

4.5.3.1. Total daily insulin dose (TDD)

Mean total daily insulin dose was significantly higher in the GlucoTab group (38.4 [\pm 28.9] vs. 10.5 [\pm 12,0]) in the conventional group.

Further secondary outcome parameters are shown in table 17.

| | GlucoTab (n=5) | ConventionalGroup (n=5) | P value |
|--|------------------|-------------------------|---------|
| TDD (mean) | 38.4 \pm 28.9 | 10.5 \pm 12.0 | <0.001 |
| Prednisolone dose (mean) | 84.7 \pm 53.3 | 98.6 \pm 59.6 | <0.001 |
| Mean survival days all patients | 100.4 \pm 63.9 | 127.4 \pm 48.8 | |
| Mean survival days exclusive 2 survivors | 80.0 \pm 51.6 | 122.3 \pm 52.0 | |
| Hospitalization days | 30.4 \pm 22.9 | 94.2 \pm 33.0 | |
| Mean hospitalization (%) | 45.3 \pm 37.8 | 77.8 \pm 18.5 | |
| Death from | | | |
| No death | 1 | 1 | |
| Infection | 2 | 2 | |
| Relapse | 1 | 1 | |
| GvHD | 1 | 1 | |

Table 17 Secondary outcome parameters

4.5. Discussion

Steroid-induced hyperglycemia is a complex side effect of glucocorticoids and a common problem appearing with an incidence of 34-56% depending on steroid dose, agent, duration of therapy and other individual conditions as comorbidity, age, et cetera (94). Patients with acute GvHD require high doses of steroids as first line immunosuppressant therapy and consecutively they are especially frequent exposed to SIHG.

General rules and guidelines for the treatment of SIHG are not available, which may be explained by the pathophysiologic complexity of the disease and the individual steroid

effect to the metabolism. However, it can be assumed that insulin therapy is the most widely established and the most sufficiently investigated treatment option especially for patients with intermittent requirements to steroids or altering steroid dosages(93, 94, 98, 157).

Our research group previously demonstrated clearly that patients with Graft-versus-Host disease face an adverse outcome when hyperglycemia appears after initiation of glucocorticoid therapy(253). However it remains still unclear whether hyperglycemia is only a marker of disease severity and a symbol of poor patient constitution or contributes itself to an adverse outcome.

Therefore we were aiming to implement glycemic management in this indicated population by using GlucoTab, a decision support system for insulin therapy recommendations. GlucoTab is usually intended to be used in non-critically ill hospitalized patients with type 2 diabetes mellitus and was primarily tested in patients with steroid-induced hyperglycemia within this study. To our knowledge this was the first study at all where an automated insulin decision support system was used in patients with steroid-induced hyperglycemia underlining the novelty of our data we hereby present.

We observed an increase of blood glucose levels during the day in both arms, which is expectable by the natural effect of steroid therapy. GlucoTab provides bolus insulin proposals which are meal dependent and distributed equally throughout the day. In this matter, we suggest higher bolus proportion in the morning in order to avoid a glucose rise after GC application. In our study we were able to improve morning glycemia by using GlucoTab. This finding can be explained by the higher amounts of basal insulin that was calculated and provided for patients in the GlucoTab group.

In this study we were able to demonstrate that GlucoTab in the treatment for SIHG is feasible and safe. Interpretation of our data is limited due to the small patient size and the non-matched population.

Larger studies are urgently needed in order to confirm our findings. However these preliminary results emphasize that research on an algorithm based tool for insulin therapy in patients with SIHG is a promising field of research activity.

4.6. Conclusion

To our knowledge we were the first ones who tested an automated algorithm based device for glucose control in hospitalized patients with SIHG.

In our pilot trial GlucoTab was safely and effectively established in GvHD patients with SIHG. Further trials including a larger population will be required in order to pave the way for technical advancement of the algorithm and routine implementation for GlucoTab in the treatment of SIHG.

4.7. Publication and presentations in scope of this study

We presented data of this study as poster at the 46th meeting of the Austrian diabetes association in November 2016 in Salzburg, Austria.

Furthermore the abstract in the scope of this prospective study is accepted as poster at the meeting of the European Association of the Study of Diabetes (EASD) which will take place in September 2017 in Lisbon, Portugal

We are planning to publish related data in Diabetes Care as “Novel Communication” after completing internal monitoring.

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APPENDIX

Presentations related to thesis:

Early Hyperglycemia after initiation of Glucocorticoid Therapy Predicts Adverse Outcome in Patients with Acute Graft-versus-Host disease

Oral presentation:

Aberer, F; Mader, JK; Stauber, M; Neumeister, P; Oulhaj, Abderrahim; Zebisch, A; Pieber, TR; Greinix, H; Sill, H; Woelfler, A; Sourij, H

Early Hyperglycemia after Initiation of Glucocorticoid Therapy Predicts Adverse Outcome in Patients with Acute Graft-vs.-Host Disease.

Diabetes.. 2017; 66(S1):A24-A25.-77th Scientific Sessions of the American Diabetes Association (ADA); JUN 9-13, 2017; San Diego, CA, USA. [Oral Communication]

Aberer, F; Stauber, MN; Mader, JK; Greinix, H; Oulhaj, A; Wölfler, A; Sourij, H
Assoziation zwischen Hyperglykämie und ungünstigem Outcome bei Patienten mit akuter oder chronischer Graft-versus-Host Erkrankung (GvHD) nach allogener Stammzelltransplantation.

Wiener Klinische Wochenschrift. 2015; 127(Supp 4):S156-S157.-43. Jahrestagung der Österreichischen Diabetes Gesellschaft (ÖDG); NOV 19-21, 2015; Salzburg, AUSTRIA. [Oral Communication]

Poster:

Aberer, F; Mader, JK; Stauber, M; Tripolt, N; Holzgruber, J; Zebisch, A; Pieber, TR; Neumeister, P; Greinix, H; Sill, H; Wölfler, A; Sourij, H

Early hyperglycaemia after initiation of glucocorticoid therapy predicts adverse outcome in patients with acute graft-versus-host disease.

4th International Hospital Diabetes Meeting. 2017; MAY 19-20, 2017; Atlanta, USA. [Poster]

Aberer, F; Stauber, M; Zebisch, A; Greinix, H; Sill, H; Mader, JK; Oulhaj, A; Pieber, TR; Wölfler, A; Sourij, H

In-Hospital Hyperglycemia Is Associated with Adverse Outcome in Patients with Acute or Chronic Graft vs. Host Disease after Hematopoietic Stem Cell Transplantation.

Diabetes. 2016; 65(S1):A390-A390.-76th Scientific Sessions of the American Diabetes Association (ADA); JUN 10-14, 2016; New Orleans, LA, USA. [Poster]

Hyperglycemia within the first month after allogeneic stem cell transplantation is an independent risk factor for overall survival in patients with acute myeloid hyperglycemia

Poster:

Aberer, F; Mader, JK; Kremser, S; Zinke-Cerwenka, W; Greinix, H; Pieber, TR; Zebisch, A; Sill, H; Oulhaj, A; Wölfler, A; Sourij, H

Hyperglykämie nach allogener Stammzelltransplantation bei Patienten mit akuter myeloischer Leukämie (AML) ist ein unabhängiger Prädiktor für das Überleben.

Wiener Klinische Wochenschrift. 2015; 127(Suppl 4):S135-S135.-43. Jahrestagung der Österreichischen Diabetes Gesellschaft (ÖDG); NOV 19-21, 2015; Salzburg, AUSTRIA. [Poster]

Aberer, F; Kremser, S; Zebisch, A; Mader, JK; Sill, H; Greinix, H; Zinke-Cerwenka, W; Pieber, TR; Oulhaj, A; Wölfler, A; Sourij, H

Hyperglycemia during the First Month after Allogeneic Hematopoietic Stem Cell Transplantation Is an Independent Risk Factor for Overall Survival in Patients with Acute Myeloid Leukemia.

Diabetes. 2016; 65(S1):A410-A411.-76th Scientific Sessions of the American Diabetes Association (ADA); JUN 10-14, 2016; New Orleans, LA, USA. [Poster]

A pilot trial to investigate feasibility and safety of an automatized decision support system (GlucoTab®) for the treatment of steroid-induced hyperglycemia in subjects with acute graft-versus-host-disease

Poster:

Aberer, F; Holzgruber, J; Tripolt, NJ; Mader, JK; Pieber, TR; Greinix, H; Zebisch, A; Woelfler, A; Sourij, H

A pilot trial to investigate efficacy and safety of an automatized decision support system for the treatment of steroid induced hyperglycemia in patients with acute Graft-versus-host disease

Diabetes.. 2017; 66(S1):A669-A669.-77th Scientific Sessions of the American Diabetes Association (ADA); JUN 9-13, 2017; San Diego, CA, USA.

Aberer, F; Holzgruber, J; Mader, JK; Wölfler, A; Greinix, H; Tripolt, N; Pieber, TR; Sourij, H

Pilotstudie zur Überprüfung der Machbarkeit und Effizienz einer normnahen Blutzuckereinstellung mittels eines automatisierten

Entscheidungsunterstützungsalgorithmus in der Insulintherapie von Patienten mit Graft-versus-host Erkrankung, Wiener klinische Wochenschrift . 2016; 44(6):410-410.-

Jahrestagung der Österreichischen Diabetes Gesellschaft (ÖDG); NOV 17,19, 2016; Salzburg . [Poster]