DIPLOMARBEIT

Changes in the biological function of peripheral mononuclear cells in diabetes mellitus - Pilot study

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Inhalt

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1 Abstract (English)

Diabetes is a potentially severe, metabolic disease affecting an increasing number of people worldwide. The prevalence of diabetes is soaring from 177 million people in 2000 to estimated 366 million in 2030. Diabetes is induced by a life-style of high-calorie nutrition, genetic predisposition and a lack of physical activity, characterized by insulin resistance and relatively impaired insulin secretion. Diabetes mellitus type 2 (DM 2), the most common form appears to be one of the main risk factors for cardiovascular disease. Monocytes represent a functionally important subpopulation of circulating leukocytes involved in local inflammatory processes under physiological as well as pathological conditions. Patients suffering from diabetes demonstrate multiple cellular and molecular alterations and defects, causing inter alia impairments of wound healing, haemostasis and atherogenesis. Macrophage migration inhibitory factor (MIF) was found to induce insulin secretion and therefore may play a role in the pathogenesis of DM 2. Additionally, MIF induces destabilization of atherosclerotic plaques and therefore may increase the risk for cardiovascular events. According to recent studies matrix metalloproteinase-9 (MMP-9) leads to increased plaque instability, resulting in stroke and myocardial infarction.

The aim of this study was the analysis of the altered function of peripheral blood mononuclear cells (PBMCs) in patients with altered glucose homeostasis. Therefore, we analysed MMP-9,its inhibitor, tissue inhibitor of metalloproteinases-1 (TIMP-1), MIF and high sensitivity C-reactive protein (hsCRP) in serum und supernatant of patients' peripheral blood mononuclear cells (PBMC) using quantitative real time PCR (qPCR) and enzyme linked immunosorbent assay (ELISA) .

Results: The cardiovascular risk was tested with hsCRP serum concentration quantification in the serum. Patients with newly diagnosed DM 2 ($p=0.0004$) and DM 2 under therapy ($p=0.0037$), had significantly elevated hsCRP levels compared to the healthy control group. However, the secreted MIF concentrations were increased exclusively after stimulation with LPS in patients with initial diagnosis in contrast to healthy donors, indicating an altered secretion pattern under inflammatory conditions. Further research has to be done, to reveal whether the proinflammatory effects of MIF play an important role in the pathogenesis of DM 2.

2 Abstract (German)

Diabetes ist eine schwere, metabolische Erkrankung, mit steigender Anzahl an Betroffenen weltweit. Die Prävalenz für Diabetes stiegt von 177 Millionen Menschen im Jahr 2000 bis zu geschätzten 366 Millionen im Jahr 2030. Diabetes wird durch einen Lebensstil von hochkalorischer Ernährung, genetischer Prädisposition und Bewegungsmangel induziert und ist charakterisiert durch Insulinresistenz und gestörter Insulinsekretion. Diabetes Typ 2, die häufigste Form erscheint als eine der Hauptrisikofaktoren für Erkrankungen des Herz-Kreislaufsystems. Monozyten repräsentieren eine funktionell wichtige Subpopulation der Leukozyten und sind in inflammatorischen, physiologischen, sowie pathologischen Prozzessen involviert. Patienten, die an Diabetes leiden, zeigen multiple zelluläre und molekulare Veränderungen und Defekte, welche u.a. Störungen der Wundheilung, Hämostase und Atherogenese verursachen. Es konnte gezeigt werden, dass Macrophage Migration Inhibitory Factor (MIF) die Insulinsekretion beeinflusst und daher auch eine Rolle in der Pathogenese des Diabetes Mellitus Typ 2 (DM 2) spielen könnte. Nach einer aktuellen Studie kann die Matrix Metalloproteinase-9 (MMP-9) und Inflammation zu einer erhöhten Plaqueinstabilität führen, was zu Herzinfarkten und Schlaganfällen führt. Daher analysierten wir MMP-9 und seinen Inhibitor, Tissue Inhibitor of Metalloproteinases-1(TIMP-1).

Das Ziel dieser Studie war die Analyse der veränderten Funktion von mononukleären Zellen des peripheren Blutes (PBMCs) bei Patienten mit veränderter Glukose-Homöostase. Dafür wurden MMP-9, TIMP-1, MIF und high sensitivity C-reactive protein (hsCRP) im Serum und Überstand der PBMCs mittels enzyme linked immunosorbent assay (ELISA) gemessen.

Ergebnis: Das kardiovaskuläre Risiko wurde mittels hsCRP Serum-Konzentration quantifiziert. Patienten mit neu diagnostiziertem DM 2 ($p=0.0004$) und Patienten mit bekanntem, therapiertem DM 2 ($p=0.0037$), zeigten signifikant erhöhte hsCRP Konzentrationen, im Vergleich zur Kontrollgruppe. Die MIF Konzentration im Überstand der PBMCs stiegen bei den Patienten mit initialer Diagnose, allerdings nur nach Stimulation mit LPS an. Was einen Hinweis auf ein verändertes Sekretionsverhalten in einem inflammatorischen Umfeld geben kann. Allerdings sind weitere in vivo und in vitro Studien notwendig, um zu beweisen, ob der proinflammatorische Effekt von MIF eine bedeutende Rolle in der Pathogenese von DM 2 hat.

3 Background

3.1 Epidemiology

Diabetes is a collective term for a group of metabolic diseases with elevated blood glucose with a soaring prevalence worldwide. [1] The different types of diabetes are characterized by hyperglycemia, impaired insulin sensitivity or insulin secretion. [1] In 2010 the prevalence of Diabetes was 284 million people globally, which implies 6.4% of the world population. [2] Predictions for 2030 indicate an increase in the prevalence to an amount of 439 million people suffering from this ailment, in other words 7.7% of the population worldwide. [2] As a continually growing economic burden, the overall costs globally reached $376 billion in 2010. [2] Especially the long-term effects of diabetes, such as myocardial infarction, chronic kidney failure, blindness, lower limb amputation and stroke, constitute a rising financial and societal issue. [2, 3] The prevalence of diabetes faces a new worldwide demography, with soaring numbers (in a period of the last 25 years increase by the factor 3-5) in asian countries as China, India, Korea, Thailand and Indonesia, depicting social change and all its implications. [2] The most afflicted regions globally are North America (prevalence 10.2%), the Middle East and North Africa (9.3%). [2]

According to the World Health Organisation in 2012 about 1,5 million people worldwide died from diabetes. [3]

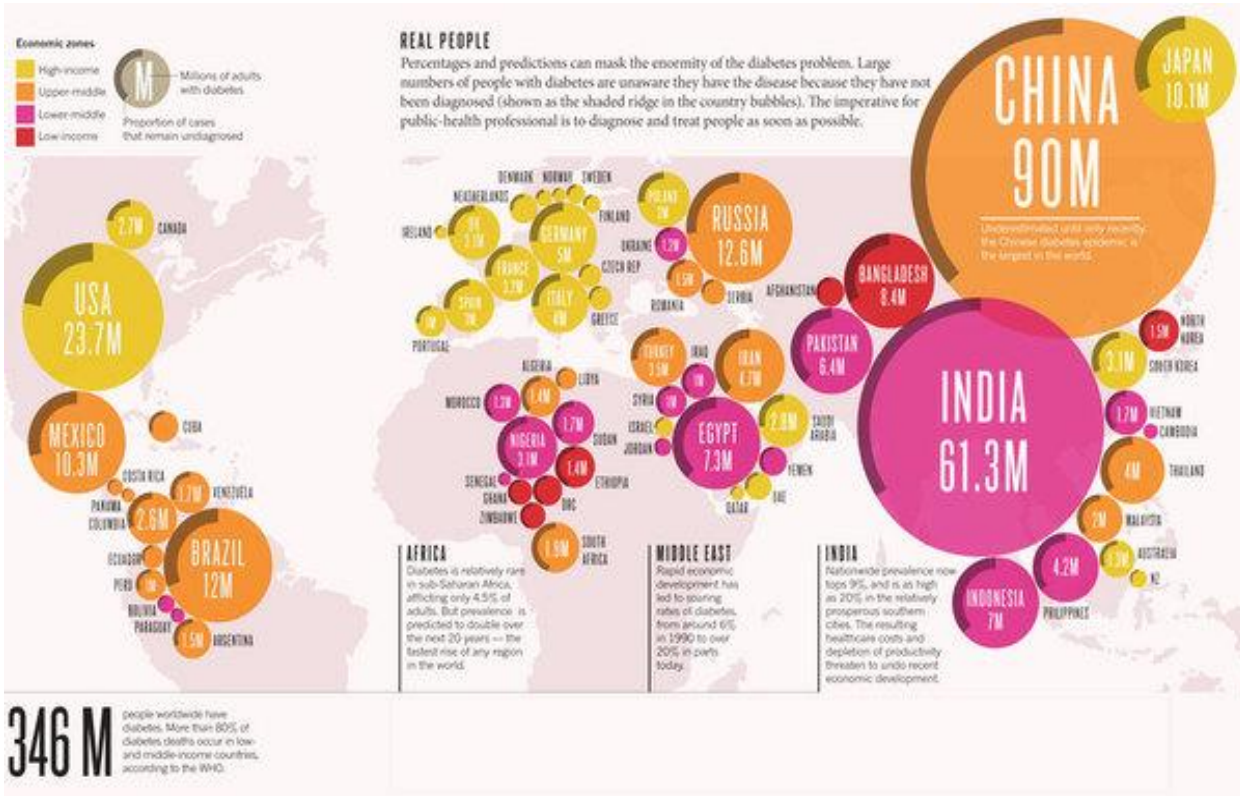

Fig. 3.1 Nature Outlook, Diabetes in numbers, Tony Scully et al. [4]

3.2 Classification and diagnosis

Diabetes can be divided into 4 major clinical classes according to their pathogenesis.

- **Type 1** is caused by the destruction of β-cells, due to auto-antibodies, creating absolute insulin deficiency. [5, 6]
- **Type 2** is based on a progression of insulin secretion defects, originated by continually increasing insulin resistance. [5, 6]
- **Type 3** is actually of multiple origins e.g dysfunction of exocrine pancreas, such as cystic fibrosis, genetic deficiencies of the β-cells or drug-induced, for example after cortisone treatment, organ transplantations or HIV treatment. [5, 6]
- The last type is **gestational diabetes (GDM)**, occurring during pregnancy and with a possible aggravation and converting into a diabetes type 2. [5, 6]

Among the type 3 forms we find MODY (Maturity Onset Diabetes of the Young), which can be subdivided in different types and LADA (Late onset Autoimmune Diabetes in the Adult). These forms of diabetes will be discussed later in this thesis. Type 3 also combines forms of diabetes induced by transplantations and cortisone. [6, 7]

3.2.1 Diagnostic threshold values

The diagnosis of diabetes mellitus may result from an elevated fasting plasma glucose of ≥ 126 mg/dl (7.0 mmol/l), a random plasma glucose above 200 mg/dl (11.1 mmol/l), an oral glucose tolerance test (OGTT), a test including measurement after the consumption of the predefined amount of 75g glucose or a HbA1c ≥ 6.5%. [5, 7-9] if reproduced, the patient is symptomatic. Another possibility is the combination of 2 tests for diagnosis. [6, 7]

3.2.1.1 Impaired fasting glucose

Patients with impaired fasting glucose (IFG), also referred to as „Prediabetes" are characterised by fasting plasma glucose levels ranging from 100 to 125 mg/dl (5.6–6.9 mmol/l) or an impaired glucose tolerance displaying 2h-glucose levels of 144-199 mg/dl (7.8–11.0 mmol/l) in an OGTT. Furthermore a HbA1c level from 5.7% up to a value of 6.4% also represents a status of increased risk for developing diabetes mellitus. [3, 6-11]

3.2.1.2 Diagnosis of gestational diabetes mellitus

For the diagnosis of gestational diabetes much lower thresholds in blood glucose levels are defined. The screening should be performed at 24-28 weeks of gestation. Two options for accomplishing diagnosis of gestational diabetes mellitus (GDM) are verified and recommended: [7, 12]

First the above mentioned OGTT with a 75g glucose uptake after at least 8 hours of fasting, according to the IADPSG consensus should be performed. Contrary to diabetes mellitus type 2 (DM 2) the necessary plasma glucose levels for diagnosis are lower in GDM. Another distinguishing feature is the importance of the plasma glucose level 1 hour after glucose uptake. In DM 2 the 1 hour glucose is not part of the measurement, whereas in GDM it is adequate for diagnosis in case of enhancement. Fasting plasma glucose ≥92 mg/dl (5.1 mmol), after 1 hour ≥180 mg/dl (10.0 mmol) and after 2 hours ≥ 153 mg/dl (8.5 mmol) are the criteria for GDM diagnosis. The second option for revealing GDM, according to the NIH consensus, is the „two step-test". [7, 12, 13]

The screening for hyperglycaemia and gestational diabetes is proceeded using a two-step process following the NIH guidelines, including a 1 hour and a 3 hour measurement or as an alternative method a one-step screening recommended by the IADPSG consensus utilizing an OGTT. [7, 14]

The one-step test is performed with an overnight fasting period of at least 8 hours, followed by a 75-g oral glucose administration and consecutive measurements of the plasma sugar level after 1 hour and 2 hours. The one step test is recommended by the IADPSG consensus.[14] The diagnosis criteria for GDM are reached at following plasma levels [7]:

Fasting period: 92 mg/dL or (5.1 mmol/L)
After 1h: 180 mg/dL or (10.0 mmol/L)
After 2h: 153 mg/dL or (8.5 mmol/L)
These criteria are determined by the IADPSG consensus [7, 14]

Two-step test differs from the one step test in a few characteristics. First of all the patients do not fast before the application of 50g glucose. If the plasma glucose level of ≥140mg/dL or (7.8mmol/L) after 1 hour is attained, the second step should be implemented. The American College of Obstetricians and Gynecologists (ACOG) advises those in charge of diabetes during pregnancy to use a lower threshold of 135 mg/dL (7.5mmol/L) to avoid under-diagnosis in ethnic minorities with higher prevalence of GDM. [7, 12, 15, 16]
The second step should be realized under fasting conditions. An OGTT with 100g glucose administration should be conducted and GDM is considered verified, if the blood glucose levels have attained the below stated thresholds. Two classifications can be distinguished: Carpenter/Coustan and from the National Diabetes Data Group (NDDG). [7, 12, 16-18]

Carpenter/Coustan
Fasting: 95 mg/dL (5.3 mmol/L)
After 1 h: 180 mg/dL (10.0 mmol/L)
After 2 h: 155 mg/dL (8.6 mmol/L)
After 3 h: 140 mg/dL (7.8 mmol/L) [7, 12, 16-19]
NDDG
Fasting: 105 mg/dL (5.8 mmol/L)
After 1 h: 190 mg/dL (10.6 mmol/L)
After 2 h: 165 mg/dL (9.2 mmol/L)
After 3 h: 145 mg/dL (8.0 mmol/L)
According to the American Diabetes Association 2014, National Diabetes Data Group (NDDG) and the NIH consensus [7, 12, 16-19]

Only one elevated value above the defined levels is adequate for diagnosis. Treatment of GDM according to these low levels of glucose shows a beneficial effect on the large-for-gestational-age (LGA) births. On the contrary the number of primary cesarean delivery could not be decreased. The two-step alternative is commonly used in the United States of America, whereas in Austria it is rarely used. [7, 13, 20].

3.2.2 Haemoglobin A1c

The haemoglobin A1c levels are the gold standard for the diagnosis of diabetes, due to advantages as higher preanalytical stability and less fluctuations during stress and infections, since it displays the average glucose level of the previous two to three months. This stability is accomplished by the 120 day life-span of erythrocytes. [7, 21] Further benefits are the reliability of HbA1c irrespective of fasting and patients' compliance. [7] Yet as every advantage may carry a disadvantage, HbA1c levels are not reliable in case of haemolysis, haemoglobinopathies, highly increased red blood cell turnover, pregnancy, blood transfusions, massive bleeding and anaemia. [7, 21] Another detriment are the higher costs for analysis, compared to fasting plasma glucose, thus it appears reasoned, that especially in developing countries HbA1c is not widely used for diagnostics. On the other hand HbA1c has a lower sensitivity as a fasting plasma glucose of $\leq$ 126 mg/dl. For final confirmation of the diagnosis, the same test should be repeated on two different days. For example if the diagnosis is ensued via random plasma glucose of > 200 mg/dl, a second measurement of the random plasma glucose should be conducted on a different day. If the cut point has been exceeded for a second time, the diagnosis is confirmed. An alternative option for diagnosis are two different tests (e.g. HbA1c and fasting plasma glucose). If both exceed the threshold the diagnosis is validated. [6-8]

3.2.3 Symptoms and risk factors

Especially in patients with DM 2, symptoms are quite vague (e.g. fatigue, polydipsia and polyuria) and evolve over a long period of time, therefore most patients seek medical care at a fulminate state with ketoacidosis or even after the appearance of long-term complications. Recapitulating it can be said, that despite frequent screening many patients suffering from diabetes remain still undiagnosed. [7]

Many studies analyzing the number of undiagnosed diabetes mellitus do not distinguish between DM type 1 or type 2, therefore the following numbers do not differentiate between

the various types. Usually the asymptomatic period of time in DM 1 is shorter, than in patients with DM 2. Due to the lack of insulin in DM 1 a fulminate disposal of symptoms can be seen at the time of diagnosis, e.g. ketoacidosis. Whereas in DM 2 the hyperglycaemia caused by insulin resistance is evolving gradually. Thus many people with type 2 remain undiagnosed for years. [1, 22]

In 2013 according to the paper from Beagly et. al. [22] a total of 174,8 million people worldwide were suffering from diabetes without diagnosis and therefore without any treatment. There is a salient contrast in the allocation of undiagnosed diabetes (UDM). The lowest percentage of UDM of all patients, has been shown in the middle income countries South and Central America with 24.1%. The highest number of UDM was 75.1% in the low income countries of Africa. These numbers are reflecting the inadequate screening frequency or screening opportunities. Based on countries, the highest level of people suffering from diabetes without diagnosis has been found in Tokelau with 20.5%. Second in line are the Marshall Islands with a prevalence of UDM of 18.9% and 16.1% has been revealed in the Federated States of Micronesia. The country displaying the lowest prevalence was Azerbaijan with 0.8%. The 10 most under diagnosed countries are all pacific islands. The countries with the highest total number (1000s) of UDM are first of all China with 53238.4 cases, ensued by India exposing 31920.0. The third highest numbers of UDM in 1000s are exhibited by the United States of America (6761.7), followed by Indonesia, the Russian Federation, Egypt, Japan, Pakistan, Brazil and Germany. [22]

Map 3.4 Number of people (20-79 years) living with diabetes who are undiagnosed, 2015

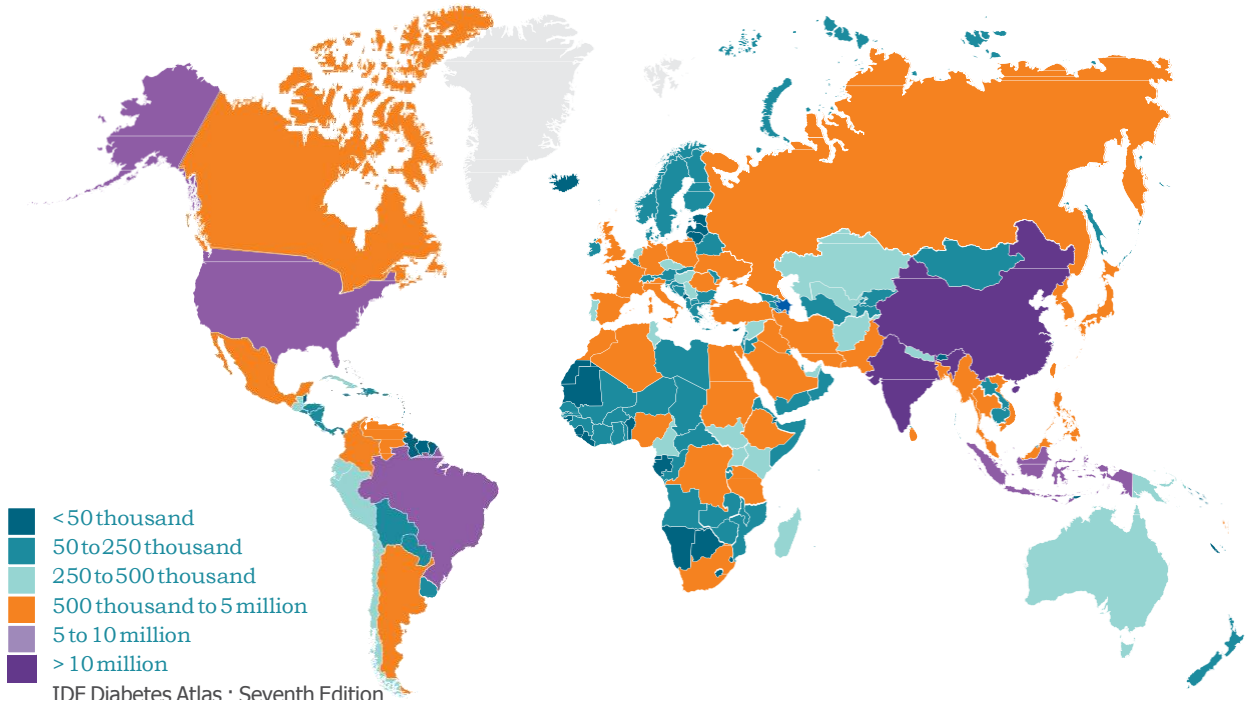

Fig. 3.1. International Diabetes Federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation, 2015. http://www.diabetesatlas.org [11]

As a result the American Diabetes Association (ADA) recommends a screening in asymptomatic persons with a Body mass index (BMI) of ≥ 25 kg/m^2 and additionally presenting one of the following risk factors: [7]

- physical inactivity
- a family history of diabetes (first-degree relative affected)
- women with previously diagnosed gestational diabetes mellitus
- high risk ethnicity
- hypertension (defined as ≥ 140 systolic and ≥ 90 diastolic blood pressure) or treated hypertension
- patients suffering from polycystic ovarian syndrome
- previous HbA1c, impaired glucose tolerance (IGT), or impaired fasting glucose (IFG) above diagnostic threshold
- patients with cardiovascular diseases [6, 7]
- patients with conditions connected to diabetes e.g. acanthosis nigricans, dyslipidemia, severe obesity, hypertension, polycystic ovarian syndrome [6, 7]

According to the American Diabetes Association 2014, [6, 7]

One of the main risk factors for diabetes mellitus is the metabolic syndrome, defined by insulin resistance and at least 2 of the following criteria: Overweight with a waist/hip ratio of > 0.90 in males and > 0.85 in females, or a BMI > 30 kg/m^2; dyslipidemia with elevated triglycerides ≥ 150mg/dL or HDL <35mg/dL (males), HDL< 39 mg/dL (females) and the last criterion is hypertension ≥ 140/90 mmHg. This definition is based on the data of the World Health Organization (WHO). [3, 23-26]

Another definition of the metabolic syndrome with glucose thresholds according to the American Diabetes Association and the American Heart Association is the Adult Treatment Panel III criteria (metabolic syndrome has been reached, if at least 3 criteria are met): a waist circumference of > 102 cm in men and > 88 cm in women. Unlike in the previous definition a threshold of a HDL cholesterol < 40 mg/dl in men and < 50 mg/dl in women, triglycerides reaching a value of ≥ 150 mg/dl, an increased blood pressure ≥ 130 mmHg and ≥ 85 mmHg and a fasting glucose level of ≥ 100 mg/dl. [26, 27]

Each of the mentioned criteria of the metabolic syndrome: impaired glucose tolerance, obesity, hypertension, dyslipidemia and hypertension can act as separate risk factor. Genetic predisposition may increase the risk of diabetes mellitus additionally. [28-30]

Individuals presenting a metabolic syndrome are facing a high risk of developing DM 2. The combination of visceral obesity, elevated triglycerides, hypertension and impaired glucose tolerance lead to insulin resistance producing a vicious circle.[31] Due to the insulin resistance, high levels of insulin are secreted, producing the sensation of increased hunger, leading to weight gain and obesity, further enhancing the insulin resistance. [31]

Criteria: metabolic syndrome	
Waist/hip ratio	>0,90 in males
	>0,85 in females
BMI	>30kg/m^2
Triglycerides	≥150mg/dL
HDL	<35mg/dL in males
	<39mg/dL in females
Hypertension	≥140/90 mmHg
Impaired fasting glucose	>100mg/dl

Table 3.1 According to the WHO [32]

3.3 Pathogenesis

3.3.1 Diabetes mellitus type 1

The polygenic genesis of Diabetes mellitus type I consists of environmental, immunologic and genetic factors. [33]

In type 1 diabetes, like in type 2 diabetes genetic predisposition plays a crucial part in the pathogenesis. This thesis is verified by a HLA DR3 and HLA DR4 positive status in more than 90% of the patients suffering from DM type 1, as well as a total of 20% of patients displaying a positive medical history of diabetes mellitus type I in their family. [31] Genes found in the HLA region encode for molecules of the major histocompatibility complex class II. These MHC II molecules are responsible for the antigen presentation in particular to helper-T cells. The activated T-lymphocytes initiate the immunologic destruction of the β-cells in the islets of Langerhans. Umpteen haplotypes of HLA loci are known to be associated with DM 1. In this regard the haplotypes: DQB1*0201, DQB1*0302 and DQA1*0301 should be emphasized, since they are found in 40% of patients suffering from DM 1.[33]

Many more additional polymorphisms predispose to DM 1 e.g. the CTLA-4 gene, PTPN22, the insulin gene (INS1) and the interleukin-2 receptor gene. Some polymorphisms actually protect from developing DM 1: DQA1*0102 and DQB1*0602. [33]

Astonishingly the risk for evolving DM 1, if the parents are suffering from it is only 3-4% and 5-15% in case of an affected sibling (these numbers may vary according to their haplotype).

As the concordance in identical twins varies between 40-60%, additional triggers are necessary for the outbreak of DM 1. [33]

The catalyst for the autoimmune process seems to be an environmental stimulus such as an infection that activates the deterioration of the pancreatic β-cells. The course of time from the trigger event until clinical symptoms and onset of diabetes may vary among individuals. This aggravates the search for possible triggers, since the intermediate time may take years.[33] Presumptive stimuli are bovine milk proteins, coxsackie virus, enteroviruses, rubella and nitrosourea compounds, but also other early nutritional influences and caesarean section.[33]

From the immunologic aspect DM 1 is caused by primary destruction of β-cells in the pancreas, due to autoimmune processes, resulting in a lack of insulin. [34] The process is caused by infiltrating lymphocytes and referred as "Insulitis". Hyperglycaemia or overt diabetes emerge, when the destruction of β-cells reaches dimensions of 70-80% and the remaining cells cannot secrete the quantity of insulin necessary for the maintenance of glycaemic homeostasis. The turnover point between impaired glucose tolerance and manifest diabetes can be triggered by a stressful event requiring elevated insulin levels, for instance infections or even puberty. After the initial appearance of DM 1 a period of good response to small doses of insulin and in some cases even withdrawal of insulin applications can be observed. This period is referred to as "honeymoon" phase with sufficient insulin secretion activity of the temporarily recovering islet cells. With progressive deterioration of the β-cells the demand of increasing insulin doses rises, until the endogenous production has vanished and all cells are destroyed. At this state the inflammatory process stagnates and the immunologic markers disperse. [33]

The autoimmune origin of DM 1 is further validated by the detection of the following antibodies in newly diagnosed patients: [31]

Autoantibodies in DM 1	Antigen
Islet cell autoantibodies (ICA)	Gangliosides
GADA	Glutamic acid decarboxylase
IA-2 antibody	Tyrosine phosphatase IA-2
Anti-insulin-autoantibodies (IAA)	(Pro)Insulin
(ZnT-8) antibody	β-cells-specific zinc transporter 8

Table 3.2 Adapted from Gerd Herold et al. [31]

The isolation of ICA is technically challenging, thus the analysis of GADA, IA-2A is more common in daily clinical practice. The diagnostic value of GADA and IA-2A combined is 90%, ZnT-8 70% and ICA 80%. IAA is a more complex marker, due to its age dependence and reaches a fluctuation range of 20-90%. In case of elevated GADA and IA-2 antibodies in healthy patients with absence of any sign of hyperglycemia, the odds of developing DM 1 in the following 5 years are about 20%. [31]

Effective trials in animal models deferring or inhibiting DM 1 by immunosuppression, blockage of cytotoxic cytokines, promoting the resistance of the islet cells towards destruction, selective T-lymphocyte subset deletion, were not successful in human applications. [33] Yet patients treated with monoclonal CD3 antibodies, application of glutamic acid decarboxylase and antibodies working on B-lymphocytes diminish the abatement of C-peptide values. [33, 34]

3.3.2 Diabetes mellitus type 2

Diabetes mellitus type 2 (DM 2) is characterized by a polygenic origin, deteriorated by environmental factors. [35] Interestingly, most diabetes predisposing polymorphisms so far described in genome wide association studies appear to impair insulin production/secretion. Typical environmental factors are a high calorie uptake and a sedentary lifestyle, implicating a major lack of physical activity. [2] Patients with DM type 2 display a failure in the secretion pattern of insulin, after glucose uptake, especially in the first phase of secretion, postprandially. On the contrary the secretion of other secretagogue agents e.g. glucagon is not impaired in patients with DM 2. Despite the hyperglycaemia constantly elevated glucagon levels aggravate the postprandial hyperglycaemia. Furthermore the hyperglycaemia is supported by the apoptosis of the β-cells, as soon as the apoptotic cells reach a total of 50%. [7, 31, 36-38]

One potential source of this pancreatic β-cell failure is the insulin resistance of the muscle and liver tissue, generating a demand for insulin exceeding far beyond physiological proportions. A higher level of insulin secretion enhances the glucose flow into β-cells of the pancreas. [35] To gain a sufficient insight in the pathogenesis of diabetes mellitus type 2 the patterns of insulin and the secretion should be explained. As already indicated insulin is created by the beta cells of the pancreas as preproinsulin, which is cleaved at the amino-terminal peptide, resulting in proinsulin. [33] The so processed proinsulin and the finished insulin resembles the insulin-like growth factors I and II (IGF). The IGFs are stimulated by

glucose uptake and also bind to the insulin receptor with a low affinity. [37, 39] The proinsulin is cleaved in a further step with the side product C-petide, both products are secreted by the β-cells, upon a stimulus of increased blood glucose levels, neurotransmitters, amino acids, incretins (especially glucagon like peptide-GLP-1) or ketones. [33] Via the Glucose transporter 1 (GLUT-1) the blood glucose is internalised and phosphorylated with the help of glucokinase, and serves as glukose-6-phosphate as supplier for the ATP-production in the mitochondria. The so generated ATP inhibits an ATP-sensitive K^+-channel causing an K^+-influx, thus subsequently leads to membrane depolarization. These electrophysiological changes lead to the opening of voltage-dependent calcium channels, which induces the secretory granules to release insulin and c-peptide into the portal venous system. [33]

The exact mechanisms that lead to peripheral insulin resistance of the muscle cells are not fully discovered, yet reactive oxygen species may play a crucial role in the process. [33] According to the study of Sakai et al. co-incubation of β-cells with a high glucose stimulation, enhanced the reactive oxygen species production by the mitochondria. Due to the reactive oxygen species (ROS) the first phase of insulin secretion was impaired. The results of the study indicated, that a reduction of reactive oxygen species, may lead to the inhibition of the development of impaired glucose tolerance. [35]

It should be mentioned, that for example the mitogenic-activated protein kinase pathway is not affected by the insulin resistance and may create via the regulation of cell growth and differentiation, atherosclerosis as a reaction to the elevated insulin levels. [33]

3.3.3 Gestational diabetes mellitus (GDM)

Gestational diabetes is caused by insulin resistance induced by various metabolic changes of advanced pregnancy. [33] Therefore hyperglycaemia appearing in the first trimester is not classified as GDM. In the United States of America 2-10% of pregnant women develop gestational diabetes. These women have a pronounced risk of evolving diabetes (35-60%). [33]

According to the American Diabetes Association a prenatal screening for undiagnosed diabetes mellitus type 2 is recommended, if risk factors e.g. family history of diabetes or severe obesity are present. During pregnancy a screening using an OGTT at 24-28 weeks should be proceeded, even in individuals without any previous symptoms of hyperglycaemia. In case of occurrence of gestational diabetes mellitus, these women should have a follow-up screening 6-12 weeks postpartum, as well as lifelong testing for hyperglycaemia and

developing diabetes in a 3 year cycle. Patients with newly diagnosed diabetes mellitus during the first trimester should not falsely be disclaimed as gestational diabetes, but classified as diabetes mellitus type 2. [7]

The complications for patients with untreated GDM are for instance, large-for-gestational-age (LGA), neonatal macrosomia or delivery difficulties e.g. shoulder dystocia. [7]

3.3.4 Other causes of diabetes

Additionally to DM 1, DM 2 and GDM, other manifold origins of diabetes exist and are summarized as "Diabetes mellitus type 3". [7]

- Genetic mutations leading to pancreatic β-cell dysfunction
    - Defects in mitochondrial DNA
    - Genetic variants coding for the protein subunits of the ATP-sensitive potassium channel
    - Mutations of insulin or proinsulin [33]
    - Monogeneic mutations leading to MODY

Variants of MODY	Mutation of
MODY 1	Hepatocyte nuclear transcription factor 4α (HNF-4α)
MODY 2	Glukokinase
MODY 3	Hepatocyte nuclear transcription factor 1α (HNF-1α)
MODY4	Insulin promoter factor-1 (IPF-1)
MODY 5	Hepatocyte nuclear transcription factor 1β (HNF-1β)
MODY 6	NeuroD1

Table 3.3 Harrison's principles of internal medicine 18th edition volume 2 page 2968-3009 [33]

- Impaired insulin action due to genetic variances of
    - Type A insulin resistance
    - Rabson-Mendenhall syndrome
    - Leprechaunism
    - Lypodystrophy syndromes

- Dysfunction or illnesses of the pancreas
    - Neoplasia
    - Cystic fibrosis
    - Pancreatitis
    - Haemochromatosis
    - Pancreatectomy
    - Fibrocalculous pancreatopathy
    - Genetically mutated carboxyl ester lipase

- Endocrine diseases
    - Cushing's syndrome
    - Acromegaly
    - Glucagonoma
    - Phaeochromocytoma
    - Hyperthyroidism
    - Somatostatinoma
    - Aldosteronoma

- Iatrogenic-, chemical- or drug-induced DM
    - Glucocorticoids
    - Pentamidine
    - Vacor (a rodenticide)
    - Nicotinic acid
    - β-adrenergic agonists
    - Diazoxide
    - Thiazides
    - Hydantoins
    - α-interferon
    - Asparaginase
    - Protease inhibitors
    - Epinephrine
    - Antipsychotics

- Immune mediated

    - Coxsackievirus
    - Cytomegalievirus
    - Congenital rubella

- Genetic syndromes
    - Down's syndrome
    - Wolfram's syndrome
    - Klinefelter's syndrome
    - Turner's syndrome
    - Huntigton's Chorea
    - Friedreich's ataxia
    - Laurence-Moon-Biedl syndrome
    - Prader-Willi syndrome
    - Porphyria
    - Myotonic dystrophy

Adapted from the American Diabetes Association 2011 and Harrison's principles of internal medicine 18th edition volume 2 [33, 40]

3.3.4.1 Maturity Onset Diabetes of the Young (MODY)

The six known variants of MODY originate from autosomal dominant, monogenic defects as previously described in the Table 3.3 Harrison's principles of internal medicine 18th edition volume 2 page 2968-3009 [33].The hepatocyte nuclear transcription factors, as the name already implies, can be found in the liver, but beyond that also in the kidneys and the pancreatic islets. There they may influence the development of the islet cells, affect the insulin secretion or regulate the preservation of the β-cell mass. The mutation of HNF-1α resulting in MODY 3 and a proceeding dysfunction of glycemic homeostasis similar to DM type 1, yet in contrary to DM 1, patients with MODY 3 are responsive to sylfonylurea. Patients suffering from MODY 5 (HNF-1β mutation) [31, 33]

3.3.4.2 Latent autoimmune diabetes of adults (LADA)

Patients with LADA typically present with signs of diabetes at the adult ranging from diabetic ketoacidosis to mild non-insulin dependent diabetes. They typically rapidly require insulin treatment and have fewer signs of the metabolic syndrome than adults with DM 2. The

diagnosis is made by laboratory test of islet cell autoantibody (ICA), glutamic acid decarboxylase autoantibody (GADA), insulinoma-associated (IA-2) autoantibody, and zinc transporter autoantibody (ZnT8) which are typically positive. LADA patients have similar immunological and genetic characteristics as DM 1 patients and DM 2. Therefore it is currently discussed whether LADA can be seen as a genetic admixture of the two forms for diabetes. It is especially not known whether LADA reflects an distinct disease entity or is part of an autoimmune continuum. [41, 42]

3.4 Complications of Diabetes mellitus

Patients suffering from diabetes mellitus type 2 display an increased risk for microvascular, and macrovascular complications, myocardial infarction, sensory neuropathy, retinopathy, stroke and an elevated all cause mortality, due to the constant hyperglycaemia. [43]

A stringent glycaemic control from the initial diagnosis onwards is essential for avoiding the development of cardiovascular disease (CVD). According to the United Kingdom Prospective Diabetes Study (UKPDS) intensive glycaemic control in initially diagnosed DM 2 patients showed a decrease in CVD events in the long term (10-year follow-up after the end of the study). The myocardial infarction rate could be reduced by 15% in the group treated with sulfonylurea or insulin after initial diagnosis and 33% with metformin. [44] The mortality has been diminished by 13% and 27% in the metformin group. [44] The UKPDS 35 trial with 4585 participants emphasizes the strong association of vascular complications to hyperglycaemia by revealing that a reduction of HbA1c of 1% can diminish the deaths caused by diabetes and its sequelae by 21%. [7, 44, 45]

3.4.1 Hypoglycaemia

When it comes to hypoglycaemia the most important aspect in the prevention is the patients' awareness for symptoms of low blood glucose. If the hypoglycaemic episodes are asymptomatic, the risk for serious neurological complications is higher, than in symptomatic patients. With regard to this lack of symptoms the self-monitoring of blood glucose gains in importance. The treatment of choice is 15-20g glucose or any kind of rapidly absorbable carbohydrates, for example a piece of dextrose or a banana. After the consumption a blood sugar measurement should be preceded. In case the low glucose level has not improved another portion of carbohydrates should be consumed. In case the glucose level has improved, a meal should be eaten by the diabetic patient, to avoid relapse of the hypoglycaemia. This

action is especially important to diminish ongoing insulin activity or in case the patient uses insulin secretagogues. An alternative treatment recommendation of the American Diabetes Association is the application of glucagon. Recurrent episodes of low blood glucose should lead to a change in the treatment regimen. Severe hypoglycaemia and a high recurrence rate bears a threat for the patient and also for the patients' environment, if they happen while driving or operating of machines. Due to neurological damages patients exhibit a higher risk for dementia and degradation in their cognitive function. Another consequence of recurrent hypoglycaemic episodes is autonomic failure, leading to impaired hormone release and in further consequence to deficient counter-regulation. Thus hypoglycaemia induces subsequent hypoglycaemia. [7, 46, 47]

According to the American Diabetes Association and The Endocrine Society the definition of a severe hypoglycaemia is an incident necessitating the assistance of another person. [7, 48] In this regard children suffering from DM 1 and older people are especially vulnerable to this condition. This may be also true as children are not capable of interpreting the symptoms correctly and taking the right action in seeking help. These events make it necessary to change the insulin application regimen, simplify it and if possible reduce the quantity of rapid-acting insulin to a minimum. Further education of the patients in the correct use of insulin, particularly in situations of decreased demand of insulin e.g. sports or fasting periods prior to medical tests or interventions, is recommended. Alterations in the alimentary habits may also play an important part in the avoidance of severe hypoglycaemia. Patients may eat a snack at night to reduce nocturnal lowering of the glucose levels. Teaching of the patients to avoid going to sleep beneath a certain glucose level threshold is equally important. [7, 48]

3.4.2 Diabetic ketoacidosis

Diabetic ketoacidosis is characterised by a hyperglycemic state, due to a relative or absolute lack of insulin. The fulminate clinical ketoacidosis is depicted by a ketonemia and metabolic acidosis. The characteristics for the diagnosis are a plasma glucose level of above 250mg/dl. A decreased pH lower than 7.30 and a reduced serum bicarbonate level < 18 (mEq/L). Ketone in the urine and the blood should be measured in every patient. The electrolytes and biochemical derangement is also a typical sign of the diabetic ketoacidosis and especially in the process of insulin-substitution it is essential to measure the K^{+-}levels, to avoid an intracellular shift. An adequate fluid therapy is also of high importance. An aggravation of the ketoacidosis leads to changes in the mental state of the patient, culminating in coma. Yet the

mortality in the diabetic ketoacidosis is mostly due to the co-morbidities or failure in insulin dosage. Due to the widespread guidelines and knowledge in the treatment of the ketoacidosis the mortality could be reduced to 17.3% in the US. [49-55]

3.5 Treatment

Diabetes care involves several clinical professions and the patients' environment, including dietitians, physicians, dentists, nurses, in some cases mental health professionals and last but not least the family of the patients. First of all I would like to emphasize the patients' responsibility and involvement in the therapy, since there are comparatively few diseases, where the patient is capable of influencing the outcome to such a high extent. The deterioration or amelioration of the illness strongly depends on the patients' knowledge and training in dealing with this ailment. DM 2 affords a high degree of patients' savoir faire and diabetes self-management education (DSME). The most important example is the behaviour during hypoglycemia, the ability of the patient to sense the symptoms, such as weakness, sweating etc. and the adequate reaction to hypoglycemia with blood sugar measurement and fast acting glucose uptake, avoidance of extensive physical activity or risky actions e.g. driving. Equally important is the patients' capability to handle hyperglycemia and the appropriate calculation of the necessary insulin dose. Life-style and dietary changes are a necessity in the appropriate diabetes care, yet the work schedule, individual or cultural patterns should be taken into consideration. Due to increased rates of infections among diabetic patients, annual influenza vaccination is recommended, as well as a pneumococcal polysaccharide immunization. Repeated vaccination makes sense, if the patients display nephrotic syndrome, diabetic nephropathy or signs of a depleted immune system (e.g. induced by immunosuppressive drugs). Clinicians should control the vaccination of hepatitis B either. This recommendation is based on the fact that the prevalence of infected persons doubles in the demographic population of diabetic patients, compared to the non-diabetic population. The cause for this effect may be the characteristic, that the Hepatitis B Virus survives even in old and dried blood stains and the higher risk for infection is induced by the wounds of patients with DM 2 after blood glucose measurement. [56] The Vaccination of Influenza leads to the diminution of hospitalizations of diabetic patients by 79%, in times of increased incidences of influenza. [7, 57]

A substantial part for the adequate treatment is the self-monitoring of blood glucose (SMBG), especially if the treatment consists of multiple-dose insulin applications or is administered

with the help of an insulin pump. Recommendations for the measurement of the blood sugar level are given before ingestion, in case of an assumed hypoglycaemia or before demanding physical activities. If hypoglycaemia has already taken place several measurements are required until the blood sugar attains a normal level. Additionally nocturnal hypoglycaemia can be averted by blood glucose measurement before going to sleep. Every once in a while postprandial glucose levels should be measured. The blood sugar measuring of the patients plays a crucial part in the treatment optimization, the patients' self management and the avoidance of hypoglycaemic episodes. [7]

3.5.1 Physical examination and follow-up

3.5.1.1 Examination

The physical examination should include at least ambulatory recording of the body mass index (BMI), therefore also weight and height. For cardiovascular risk factor calculation it is also very important to measure the blood pressure on a regular basis. The thyroid function should be observed by palpation and the TSH parameter, when it comes to patients with dyslipidemia, DM type I and women aged 50 or older. To avoid discovering diabetic retinopathy at a late stage an annual fundoscopic screening is recommended by the American Diabetes Association (ADA). [7] Every person affected should have a nutritional consultation at a specialized dietitian. A skin inspection should be proceeded on the one hand to control if any lipohypertrophy occurred due to insulin injection or if signs of acanthosis nigricans are visible. On the other hand special attention should be paid on the feet and their perfusion of diabetic patients. Diabetic neuropathy, microvascular and macrovascular angiopathy (peripheral artery disease) and a possible resulting wound healing disorder should be averted by examining the dorsalis pedis as well as the posterior tibial pulse, achilles and patellar reflexes, monofilament sensation, sensitivity for vibration and proprioception. [7]

Subsequent laboratory parameters should be monitored annually: triglycerides, LDL, HDL cholesterol, liver function parameters to reveal any abnormality e.g. steatosis hepatis. To test if any nephropathy is emerging the serum creatinine, albumin-to-creatinine ratio in the spot urine and the calculated glomerular filtration rate (GFR) should be analyzed. The above mentioned TSH and HbA1c, whereas the HbA1c is very important for planning the treatment schedule and should not be older than 2-3 months. [7]

3.5.1.2 Anamnesis

First of all the question for the initial diagnosis is essential and provides a rich source of further questions for the treatment schedule. If the initial diagnosis for a patient with DM 2 was 20 years ago the question for long-term complications has a different emphasis, than for a middle-aged patient newly diagnosed with DM 2, 2 years ago. The age of the initial diagnosis is equally important and the status of the auto antibodies in DM 1 or LADA or analysis for gene mutations resulting in MODY. Of particular importance is the life-style of the patient in concern of the alimentary habits, physical activity, BMI and any abnormality in the growth pattern during childhood or adolescence. Another simple but important question is the patients' education regarding his own disease and possible alimentary changes discussed with a dietitian. The previous and current treatment is vital for the further treatment decisions, especially when it comes to drug incompatibilities or insufficient therapies. The records of the self-monitoring the patient's blood glucose and HbA1c notations are one of the most informative basis for the choice of treatment schedule. Errors in therapy and their cause such as diabetic ketoacidosis or hypoglycemic episodes should be assessed and the consciousness for these blood glucose derailments inquired and promoted. The long term consequences and complications enquired concerning microvascular, macrovascular (e.g. peripheral artery disease), periodontal, autonomic dysfunctions for instance gastroparesis or erectile dysfunction and psychosocial problems. [7]

3.5.2 Glycemic target values

According to the American Diabetes Association, for the treatment surveillance the HbA1c and the plasma glucose are of particular significance for reaching an adequate metabolic control and reduction of the risk factors for long term complications. [7]

As mentioned above the HbA1c should not be measured with a frequency less than 3 months. Unless the patient is in a critical, unstable phase of its treatment, for instance pregnant women suffering from type 1 diabetes. In patients displaying a good response to the therapy analysis twice a year are sufficient. On the contrary in patients with inadequate response to therapy HbA1c should be performed trimestrially. In patients vulnerable to strong fluctuations of blood glucose values, the best approach for planning the treatment schedule is a combination of HbA1c and self-monitoring of blood glucose (SMBG). In case of HbA1c levels are at variance with the self measured blood glucose, the proper handling of the measurement device should be validated or a haemoglobinopathy excluded. [7]

The glycemic goal for reduction of the likelihood of macrovascular complications in adults is a HbA1c less or equal 7%. Exceptional cases with a target value of ≤ 8% HbA1c are patients with severe hypoglycemic episodes in their anamnesis or already existing grave microvascular and/or macrovascular complications, reduced life expectancy, poor general condition, multiple diseases and long-lasting diabetes or insulin treatment. [7]

3.5.3 Hypertension

Patients showing an elevated blood pressure of more than 140 mmHg of systolic pressure and more than 90 mmHg of diastolic pressure should be treated with changes in their lifestyle, as well as pharmacological treatment. Control of blood pressure, especially in diabetic patients, is an effective method to prevent long term complications, such as cardiovascular disease and microvascular complications. Hypertension should be verified by measurement on two separate days. If a White-Coat induced hypertension is suspected, a 24h blood pressure monitoring may reveal the actual pressure levels. For correct measurements the adequate size of the blood pressure cuff has to be chosen. The mentioned lifestyle alterations include increased physical activity, weight loss, diminution of alcohol consumption, reduction of sodium-uptake and increased potassium-uptake. Antihypertensive pharmacological therapy should consist of an angiotensin-converting-enzyme (ACE) inhibitor, or alternatively an angiotensin receptor blocker (ARB). Usually monotherapy is insufficient to reach the recommended treatment goal of less than 140/80 mmHg. A meta-analysis comparing blood pressure levels to DM 2 complications, revealed, that 130-135 of systolic pressure was adequate. A more strict regulation of <130mmHg was associated with a lower rate of strokes, a decrease of mortality of 10% and a later onset and slower aggravation of albuminuria. [7, 58] Multiple combinations are possible with β-blockers, calcium channel blockers or diuretic agents and are verified to have a positive effect on the decrease of cardiovascular complications. A pharmacological treatment of hypertension is usually based on changes in electrolyte shifting and therefore a routinely control of potassium, the estimated glomerular filtration rate and serum creatinine should be examined. [59] Pregnancy requires special attention to the blood pressure, on the one hand due to contraindication of ARBs and ACE inhibitors and on the other hand, due to the lower treatment aim of 110-129 mmHg systolic pressure and 65-79 mmHg diastolic pressure. Alternatively to the ACE-inhibitors and the ARBs, several pharmacological drugs such as diltiazem, methyldopa, clonidine, prazosin or labetalol are indicated and verified as safe during pregnancy. The origin of hypertension differs between the various types of diabetes. Whereas in DM 1 the elevated pressure levels

are a consequence of the diabetic nephropathy, in DM 2 the hypertension is usually part of a multitude of cardiovascular risk factors associated with adiposity, high caloric and sodium-rich alimentation and a lack of physical exercise. A systolic blood pressure exceeding 120mmHg may be a prognostic marker for end-stage renal disease, after years of DM 2. [7, 60-63]

3.5.4 Dyslipidemia

Patients with DM 2 display an increased risk in developing lipid abnormalities. Vice versa many patients under statin therapy have a higher risk for evolving an impaired fasting glucose or diabetes mellitus. [64, 65] According to a meta-analysis comparing 14 randomized studies, including 18.000 patients, therapeutical use of a statin resulted in a 9% decrease of all-cause mortality and 13% decrease in mortality caused by vascular complication, for every reduced mmol/l. [7, 66] Once in a year a screening for elevated fasting lipids should be proceeded. In patients with a low risk for dyslipidemia, displaying a lipid profile of HDL > 50, LDL < 100 and triglycerids <150 mg/dl, a screening rate of once in two years is sufficient. The first-line therapy is a life-style modification of the patient. These changes in the habits of the patients include weight loss, increased physical activity, diminution of trans fat, saturated fat and cholesterol consumption. Alimentation should include a higher part of plant stenols, viscous fiber, and n-3 fatty acids. [7] Statin therapy is indicated as second line therapy, after insufficient amelioration after life-style changes. One exception is severe hypertriglyceridemia, especially if the level is high enough to induce pancreatitis. Another exception are diabetic patients with a history of a cardiovascular complication, or aged above 40 and additional risk factors of CVD. [7, 66-68]

The target goal for lipid levels in patients lacking any CVD in their medical history is a LDL <100mg/dl. Patients with events of CVD in their history may benefit from a lower LDL level of <70mg/dl. In case the target levels cannot be achieved with the highest dose tolerated by the patient, a decrease of 30-40% from baseline levels are adequate. Triglyceride levels should not exceed 150mg/dl. Yet the lipid therapy is based on the evaluation of LDL cholesterol. A two-drug combination with a fibrate has failed to show additional effects on the decrease of CVD. [69] Addition of fibrates or niacin to a statin therapy may decrease levels of all lipid fractions, yet this positive effect has a high price of a relevantly elevated risk for rhabdomyolysis, renal insufficiency and increased transaminase levels. [7, 10, 70, 71]

3.5.5 Cardiovascular disease

Prevention of cardiovascular disease in diabetic patients is way more important, than the screening for it. Thus as above mentioned, the strict control of lipid-levels, hypertension, smoking, albuminuria or other risk factors for complications of the cardiovascular system (e.g. a positive family history of CVD) is of high priority. [7, 72] Cigarette smoking is one of the major risk factors for developing cardiovascular complications, which induces further development of risk factors. A study enrolled diabetic patients at initial diagnosis and analysed the effects of smoking. Smoking cessation leads to improved metabolic parameters, lower blood pressure levels and decreased albuminuria after 1 year. [73] After a myocardial infarction the recommendation of the American Diabetes Association is a continuing β-blocker therapy for 2 years, it may be prolonged, but 2 years are the minimum duration. Screening for coronary artery disease in diabetic patients is recommended, if patients display cardiac symptoms and/or pathological ECG. [7, 74]

3.5.5.1 Anticoagulant medication

Anticoagulant medication (e.g. Aspirin 75–162 mg/day or ThromboAss) is recommended for diabetic patients displaying an elevated risk for CVD. The application of antiplatelet drugs should be prescribed for patients with a 10 year risk of developing a CVD of more than 10%. This indication adresses men over 50 years and women over 60 years with one or more risk factors for complications of the cardiovascular system or an CVD event in their medical or family history. Alternatively if aspirin is not tolerated, due to allergy or contraindications (e.g. Reye-syndrome in patients under 21 years of age) clopidogrel is recommended. In case an acute coronary syndrome has occurred, a dual antiplatelet drug application for one year should be proceeded. Possible combinants are clopidogrel or ticagrelor, regardless of whether a percutaneous coronary intervention was performed. Prasugrel is only indicated after percutaneous coronary intervention of an acute coronary syndrome. The effect of a decreasing cardiovascular mortality of aspirin as secondary prevention has been verified, yet the data for a preventive indication for patients without CVD events are widely discussed. [7, 75, 76]

A meta-analysis of 6 studies combining a total of 95.000 participants (only 4000 diabetic patients) revealed a 12% diminution in the risk for vascular complications (Antithrombotic Trialists' collaborators). An essential difference in the effect of antiplatelet therapy between men and women was shown. Whereas aspirin was able to diminish CVD events in men, this positive outcome was not valid for women. On the contrary the incidence of stroke was

reduced in the female study population, but not in the male participants.[75] Summarizing the data, the most ferocious side effects of antiplatelet medication is gastrointestinal bleeding with a prevalence of 1-5 out of 1000 patients. For patients with a risk higher than 1% for CVD, the rate of averted CVD events equals or outweighs the possible bleedings, induced by anticoagulant medication. According to an agreement of 2010 of the American Diabetes Association, the American College of Cardiology Foundation and the American Heart Association, a dose of 75–162 mg/day of aspirin is reasonable and adequate as primary prevention for diabetic patients with a risk exceeding 10% for CVD events in 10 years, if they lack any bleeding tendency. [7, 72, 76]

3.5.6 Diabetic nephropathy

The prevention or inhibiting of the aggravation of diabetes-induced nephropathy is closely related to the blood glucose control and hypertension treatment. [10, 45, 60, 77, 78] The screening for nephropathy is conducted with the albumin excretion in the urine. The recommended screening test is the albumin-to-creatinine ratio in a random urine sample. [79, 80] The analysis of urinary albumin alone is vulnerable to measurement errors, due to exsiccosis, hypertension, exercise within 24h prior to the evaluation, infections and resulting fever and last but not least hyperglycaemia. It is crucial to test the albuminuria in every DM 2 patient, due to their long asymptomatic period prior to diagnosis and in every patient with DM 1 with a diabetic medical history of more than 5 years. Below a level of <30mg of albuminuria in 24 hours, a medical therapy with neither angiotensin receptor blockers or ACE inhibitors are indicated, as long as no hypertension is verifiable. In moderate albuminuria ranging from >30mg to <300mg per 24h and severe albuminuria >300mg a pharmaceutical therapy is recommended by the American Diabetes Association. The common definitions "microalbuminuria" and "macroalbuminuria" are not mentioned on purpose, according to new trends in medicine to avoid the intermittent character and emphasize the permanence of elevated urinary albumin excretion. Adequate drugs are ACE inhibitors or ARBs, yet a combination is not indicated, due to the higher risk of adverse events, that outnumber the benefit of reduced rates of urinary albumin excretion. [81] Alternatively β-blockers, diuretics or calcium channel blockers can be administered, if ACE inhibitors are contraindicated [7, 81-87]

In case the origin of the kidney disease is not completely evidenced, or if aggravation including possible dialysis takes place, an expert in nephrology should be consulted. A

permanent moderate albuminuria not exceeding 300mg per day is an accepted risk factor for developing CVD and already considered as a mild form of nephropathy in patients suffering from DM 1. [88-90] Yet unlike the common aggravation from an albuminuria of >300mg to end-stage renal disease, patients with a moderate level of urine albumin excretion from 30-299mg display an amelioration to normal renal function in nearly 40% of patients with DM 1. Another 40% maintain at this level, without diminution of the kidney function at least for 5-10 years. [7, 83, 91-95]

Stages of chronic kidney disease	GFR (mL/min/1.73 m2 body surface area)
Stage 1	≥90 GFR
Stage 2	60–89
Stage 3	30–59
Stage 4	15–29
Stage 5	<15(kidney failure)

Table 3.4 Stages of chronic kidney disease, adapted from Levey et al. [7, 80]

3.5.7 Treatment in diabetes mellitus type 1

Most important for patients suffering from diabetes mellitus type 1 is the information and training of the correct usage of basal and prandial insulin. Therefore it is crucial to measure the blood glucose levels preprandially and acting accordingly. The Diabetes control and complications trial (DCCT) highlights the necessity of strict glycemic control and insulin application 3-4 times per day, or a continuous subcutaneous insulin infusion e.g. insulin pumps. [96] The insulin application, if not given continuously should be divided in basal and prandial insulin to simulate a nearly normal insulin secretion pattern of the pancreas, to the highest possible level. To achieve this goal, a precise matching of preprandial glucose, insulin dosage and food uptake/ consume of carbohydrates should be conducted. Of equal importance is the adjustment of physical activity and also physical stress, such as febrile infections etc. An injection rate of at least 3-4 times is recommended by the DCCT study to achieve adequate glucose levels and a decrease in long term complications, especially microvascular deficiencies. Yet the avoidance of hypoglycemia is of equal importance as a strict glycemic control, therefore insulin analogues offer an important advantage, compared to conventional insulin. When it comes to hypoglycemia the choice of the adequate insulin pump should consider hypoglycemic patterns, e.g. nocturnal hypoglycemia, a lack of symptoms and

awareness of the patient for a low glucose level. In these cases a pump with a low glucose sensor and a fixated treshold controlled release of insulin should be advised. [7, 96-98]

Comparing applications of insulin/insulin analogues and insulin pumps, no evident differences in the HbA1c levels could be shown so far. [99] Yet when it comes to nocturnal hypoglycemia a sensoring, threshold-suspended insulin pump can decrease the rate of hypoglycemic episodes, whereas the achieved level of HbA1c does not differ significantly from the values of patients using intensified insulin therapy [97]. Equally important is the compliance and support of the patients' family. [7]

Due to the autoimmune aspect of DM 1 the screening for further diseases caused by immunologic processes is eminently important. In this aspect a screening for autoimmune processes of the thyroid, celiac disease and a lack of vitamin B12 is necessary, due to a strong correlation in the prevalence of these diseases with DM 1 has been revealed. [7]

3.5.8 Medical therapy of diabetes mellitus type 2

According to the guidelines of the American Diabetes Association [6, 7] the basis for pharmacological treatment of DM 2 is metformin. If no allergies or contraindications inhibit the use of metformin, it is recommended as initial medication. Yet after the initial diagnosis of symptomatic patients, displaying high glucose and HbA1c levels insulin therapy at the beginning should be taken into consideration. In case Insulin therapy is not sufficient initially, additional oral anti-diabetics can be added. [6, 7]

Metformin dosage should be increased in case the target goal of HbA1c cannot be achieved within 3 months. In case the metformin therapy has reached the maximum and the treatment goals are still not achieved, a second oral antidiabetic drug can be started e.g. sulfonylurea, Thiazolidinedione or a DPP-4 inhibitor. Alternatively a GLP-1 receptor agonist, SGLT-2 inhibitors or insulin can be used as additional medication to metformin. Due to the pathogenesis of DM 2 and the inevitable deterioration of insulin sensitivity and hyperglycaemia, additional drug applications are quite inevitable. Therefore it is important to ascertain the ideal combination of antidiabetics for every patient individually. This decision should be based on the side effects, pharmacological characteristics, financial aspects, risk for hypoglycaemia, possible weight loss and the individual risk for comorbidities and last but not least the patients' personal preferences. In case the two drug combination is not leading to a reduction in HbA1c, a change of the second agent, next to metformin is necessary. Every class of antidiabetic medication (except for insulin) leads to an average HbA1c reduction of

0,9 to 1,1%. [100] In case a combination of metformin and all of the above mentioned drugs fails in reaching the treatment goals, a three drug medication should be initialised. The follow-up consultation should be realised every 3-6 months after changes in the drug prescription. Should a three drug combination fail in lowering the plasma glucose to an adequate level, a more complex insulin application schedule should be implemented. This schedule is based on multiple insulin doses and a combination of basal insulin and a rapid acting insulin. Optionally an additional oral antidiabetic agent can be added (or a GLP-1 receptor agonist). [6, 7, 100]

No matter which pharmacological agents the patients get, lifestyle changes, physical activity, weight reduction (in case of obesity) and a healthy diet is recommended for every patient. In order to achieve an effective change in the alimentary habits of the patients, a nutrition counselling by a dietitian, conversant with diabetes is strongly recommended. Alcohol bears a risk of delayed hypoglycaemic episodes for diabetic patients, thus the patients should be informed about the consequents of alcohol. When it comes to physical activity the recommendation for patients suffering from DM 2 is a weekly amount of 150 min of moderate training, three days a week. The exercising routine should be constant with a maximum of 2 days in a row without training. On average constant and structured exercise, if performed for 8 consecutive weeks show a HbA1c reduction of 0,66%. [6, 7, 101]

Patients suffering from diabetes have a high rate of self management responsibility in coping with their chronic disease. To guarantee an improvement in glucose levels and a reduction in long term complication the psychosocial wellbeing is of high interest for the medical management. Psychological and social problems as depression, financial problems, eating disorders, support of the social environment and a handicap of the cognitive abilities should be taken into consideration. For example 20-25% of diabetic patients suffer from depression, which can raise the overall mortality.[7, 102-104] Diabetes as a chronic, life-long disease also requires management of intermittent trauma, infections or surgery. Thus the treatment of diabetes may be varied according to actual situations and be adapted to comorbidities or iatrogen changes e.g. cortisone, which leads to hyperglycaemia. Febrile infections necessitate an insulin adaption, as well as the fact that metformin should be paused prior to a surgical intervention, due to the risk of ketoacidosis. The treatment regimen should be adapted in situations of aggravated glycemic control (e.g. trauma) and the frequency of blood glucose values, urine and blood ketone measurement should be increased in times of elevated physical stress. Under the circumstances of illnesses, patients using solely oral antidiabetic agents may

need temporary insulin application. Special attention should be paid on the fluid in hyperglycemic episodes. Dehydration and nonketotic hyperosmolar state during infections are common reasons for hospitalization of patients with diabetes. [7]

3.6 Diabetes and Inflammation

As shown in previous studies, the murine and human adipose tissue contain a high number of macrophages. These macrophages play a role in insulin resistance and the pathogenesis of diabetes. [105-107] The macrophages as part of the innate immune system secrete tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), cytokines that have been proved, to augment insulin resistance in adipocytes. [108-110] Taking the adaptive immune system into consideration, a study in mice with absent T- or B-lymphocytes, severe combined immunodeficiency (SCID), revealed that mice with impaired adaptive immune system developed a decreased insulin sensitivity and diminished glucose tolerance, under high fat diet and control diet either. Therefore also an effect on the glucose homeostasis can be identified, as the lack of B- and T-lymphocytes could not inhibit the progression of impaired glucose levels. [111] Oxidative stress also affects the development of insulin resistance and diabetes mellitus type 2. [112] A study measuring the risk for diabetes, has revealed the association between elevated markers of oxidative stress and DM 2. [113] MIF, as an inflammatory cytokine may also play an important role in the evolvement of glucose intolerance as it is involved in the glucose metabolism[114] [111, 113, 115-120] [121]

3.7 Peripheral mononuclear cells

Peripheral blood mononuclear cells (PBMCs) are a cell population demonstrating significant importance in the immune system, consisting of monocytes and lymphocytes. Both monocytes and lymphocytes play a crucial role in inflammation, particularly by secretion of cytokines and chemokines affecting migration, vascular adhesion and coagulation. Monocytes as an example secrete among various other cytokines interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), activate multiple endothelial cells (promoting them to secrete adhesion molecules like vascular cell adhesion molecule-1,VCAM-1) and immune cells by presenting antigens to T-cells. Lymphocytes also activate other immune cells (for instance macrophages with IFN-γ, granulocyte macrophage-colony stimulating factor GM-CSF or TNF-α), release proinflammatory cytokines like interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and IL-6 or cytotoxins produced by CD8 T-cells e.g. Perforin, Granzymes or Granulysin.

Activated PBMCs appear to be an important factor in the pathogenesis of various diseases, such as atherosclerosis, asthma, diabetes, cancer and congestive heart failure. MIF is secreted by monocytes, macrophages and among various others, even cells of the anterior pituitary gland. MIF is a proinflammatory cytokine, that regulates the innate and adaptive immune system and induces the activation of macrophages and T-lymphocytes. The stimulation of MMPs is enhanced by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) secreted by monocytes and macrophages. In a study of White et al. was shown, that PBMCs regulated the expression of MID and MMP-9. The inhibition of MIF lead to lower levels of MMP-9 expression. MMP-9 was also induced by IL-1β. [122-127]

3.8 Macrophage migration inhibitory factor (MIF)

Macrophage migration inhibitory factor is a well-known pleiotropic actor of innate immunity. The strong link to the glucose metabolism has been shown, in various studies. [127-130] On the one hand a high glucose level leads to an enhanced MIF expression, on the other hand MIF may contribute to the counter regulation by supporting the insulin secretion. This mechanism may play a role especially at the beginning of the development of diabetes mellitus, when the pancreatic beta cells start to fail, or an evolving insulin resistance demands a higher level of insulin for maintaining sufficient values. MIF can be secreted by peripheral mononuclear cells (PBMCs) and also from adipocytes, therefore a correlation between obesity and increased MIF levels is not surprising. [129-135]

Due to the secretion by PBMCs the question how a subclinical, chronic activation of the immune system effects the secretion patterns in diabetes mellitus, or if an additional inflammatory impulse is necessary to release the full impact of MIF on the pathogenesis of impaired fasting glucose and diabetes mellitus. Under physiological conditions, the regulation of insulin secretion of MIF has no sign of pathology, yet under the circumstances of a chronic systemic inflammatory environment and altered glucose levels, this enhanced insulin release may lead to apoptosis of the pancreatic beta cells. [129, 136-140] Further questions arise when it comes to cause and effect in concern to diabetes mellitus. Is the elevated MIF level the cause or a consequence of diabetes mellitus. In a study with high-fructose diet for rats, with the aim to induce impaired glucose homeostasis, the analysed systemic MIF levels in the plasma depicted a trend to higher concentrations and significantly higher levels in the adipose tissue of the fructose-fed animals. [127-136]

In the study of Herder et al. investigating the study population of KORA Augsburg, a cox proportional hazard model revealed a correlation of an increased risk for diabetes mellitus type 2 and systemic MIF levels in women. However this effect could not be seen in the male population, actually the opposite was the case. [128, 129, 134]

MIF plays a crucial role in inflammatory pathologies such as sepsis, various types of cancer and diseases with autoimmune origin. [141, 142] MIF has a strong impact on the cell migration by binding to the receptor CD74 and regulates TNF-α and interferon γ and acts through this pathway as a proinflammatory cytokine. In combination with glucocorticoids, MIF is even able to adhere their antiinflammatory effect. Ironically MIF is induced by low levels of glucocorticoids. [133, 141-145]

MIF is also associated with multiple complex coronary lesions. The study from Hao et al. compared MIF serum levels from patients with simple and complex coronary lesions. The lesions were angiographically verified and a total of 308 participants were enrolled. The conclusion of the study was a significantly higher concentration of MIF in the patients displaying complex lesions. The study population with stable angina pectoris presented higher MIF serum values than the healthy control group. This may indicate a role of inflammatory markers (MIF) in plaque instability and vulnerability in plaque rupture. [146, 147]

MIF is also a strong regulator of proinflammatory cytokines, such as MCP-1, TNF-α VCAM1, IL-6, ICAM1 and MCP-1. These cytokines additionally play a role in the genesis of and destabilization of atherosclerotic plaques. The possible coherence of MIF and atherosclerotic plaques was also basis for the research of Sun et.al.. He induced diabetes in apolipoprotein E-deficient mice with streptozotocin, a drug used in the therapy of insulinoma. One third of the diabetic mice received an adenovirus-mediated MIF interference injection, another third Ad-enhanced green fluorescent protein (EGFP) injections and the last third was the diabetic control group and injections of normal saline were applied. A group without induction of diabetes made up the control group. After the sacrifice of the mice, the plaque measurements were controlled in the histological The results in the MIF-gene silenced mice showed a decrease in the ratio of the plaque area/total cross sectional are of the vessel wall compared to the control group in the aortic root, as well as the abdominal aorta and the carotid artery. [148] Thus MIF might play an important role in the development of plaques, when it comes to diabetic patients. Furthermore the plaque instability index was diminished (Oil Red O+ area plus MAC+ area)/(a-SMA+ area plus collagen I+ area) significantly in the mice after the gene silencing of MIF and the Collagen I/III ratio was reduced. [148] Summarizing it can

be said, that in the MIF-gene silenced mice, formation of atherosclerotic plaques was evidently reduced and the plaques were stabilized, due to the diminution of inflammatory cytokines usually induced by MIF. [147, 148]

3.9 hsCRP

hsCRP is an inflammatory marker, that indicates the cardiovascular risk of patients, similar to known risk factors, for example as mentioned above hypertension and dyslipidemia. [149, 150]

The origin of atherosclerotic plaques and thrombosis is not restricted to the haemostasis and thrombotic system. The immune system and in this context especially the mononuclear cells, the secreted cytokines and the vessel endothelium play an important part in the development of atherosclerotic lesions. [151] The risk for cardiovascular events, such as myocardial infarction and stroke is linked to changes in the immune system and the secretion patterns of mononuclear cells and these distinctions are not fully discovered yet. The marker hsCRP is used in the clinical daily routine for the probability assessment in primary prevention confirming the indication for the start of statin therapy in the US. [152] A study by Ridker et al. revealed, that the chronic low-grade inflammation plays a role in the cardiovascular rsik and is detected by hsCRP. Aspirin with its antiinflammatory effect, may reduce the vascular event risk not only via the anticoagulant effect, but by the reduction of the inflammation. [153] The marker hsCRP is more specific for plaque instability and thromboembolic events, than to the mere presence of atherosclerosis. The predictive value is comparable to LDL cholesterol. [149, 152, 154]

3.10 MMP-9

Matrix Metalloproteinases (MMP) are endopeptidases with a total of 24 known, various subtypes. To avoid uncontrolled cleavage, most MMPs circulate in the vascular system as inactive proMMPs. An activation is necessary after the secretion. The stimulation of MMPs is enhanced by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) secreted by monocytes and macrophages. As a result the cleavage of extracellular matrix is induced by the stimulated MMPs, leading to elevated migration of inflammatory cells into the vascular vessel wall and vascular tissue. Therefore MMPs play a crucial part in the vascular remodelling in an inflammatory environment. [126, 155, 156]

3.11 Tissue inhibitors of metalloproteinases (TIMP-1)

The main function of the tissue inhibitors of the metalloproteinases (TIMPs) is the regulation of the, as the name already indicates, matrix metalloproteinases and disintegrin metalloproteinases (ADAMS) or disintegrin metalloproteinases with thrombospondin motifs (ADAMTS) in the remodelling processes of the extracellular matrix. Aside from this main task TIMPs play a role as signaling molecules. In the last decades more and more functions of the TIMPs have been discovered, among them apoptosis, carcinogenic effects, angiogenesis and cell growth. These regulatory effects are implemented by a cytokine-like behaviour of the TIMPs and binding to specific receptors. Four different types of TIMPs, are established in the literature (TIMP-1, TIMP-2, TIMP-3, TIMP-4). From these TIMP-1 and TIMP-2 are the most conversant proteins. [157-161] TIMP-1 exists in a soluble form and also as a cell surface protein. TIMP-1 was able to induce cell growth in multiple cell lines, e.g. fibroblasts, epithelial cells, keratinocytes, chondrocytes and various cancer cell lines. TIMP-1 was discovered to be elevated in patients with heart failure and also rising with disease aggravation, indicating a possible utility as biomarker for disease severity. Increased levels of TIMP-1 were also found in patients with ischemic heart failure. [162] [163]

4 Materials and Methods

4.1 Study design

The study was conducted as a double centre explorative basic science pilot-study. The contributing centres were the Department of Endocrinology, division of Internal Medicine III, of the Medical University of Vienna and the "Wiener Gebietskrankenkasse-Gesundheitszentrum Wien-Süd" (WGKK). Additionally Dr. Maria Gremmel was informing patients about the opportunity of the study at the Department of Endocrinology, division of Internal Medicine. The rationale of the study was to evaluate changes in gene expression and cytokine secretion pattern in peripheral blood mononuclear cells (PBMCs) in patients with impaired fasting glucose, patients with fulminate diabetes mellitus type 2 under therapy, patients at initial diagnosis without therapy and healthy volunteers. Ethical approval from the institutional review board on human research of the Medical University of Vienna was obtained (Voting Nr. 1510/2013).

The laboratory work and scientific measurements were performed at the Department of Surgery (surgical research facilities) of the Medical University of Vienna and with the support of the Christian Doppler Laboratory for Diagnosis and Regeneration of Cardiac and Thoracic diseases.

4.2 Aim of the Study

The aims of this study were:
(a) the analysis of changes in the physiological function, gene expression and secretion pattern of PBMCs in patients with diabetes
(b) the identification of possible correlations with disease severity.

Fibrinolytic dysfunction mediates the increased risk of coronary artery disease in individuals with metabolic syndrome [164, 165]. Adipose tissue induces thrombocyte activation by the production of adipokines, of which some, such as leptin and adiponectin have been shown to directly interfere with platelet function. Increased adipose tissue mass induces insulin resistance and systemic low-grade inflammation, also affecting platelet function [164-166].

Beside cells of the adipose tissue, circulating mononuclear cells seem to contribute to enhanced coagulation in diabetic patients as well. Changes in insulin or blood glucose levels may result in a pro-inflammatory phenotype and altered secretion patterns of PBMCs. However cross-talk between platelets and monocytes is now regarded as a crucial pathophysiological mechanism linking thrombosis and inflammation. In vitro studies show that formation of monocyte-platelet aggregates increases cytokine and prostanoid production by monocytes [166]. According to the data of a recently conducted study of our research group, we hypothesized that the secretory product of PBMCs was underestimated in disease development by inducing hepatic insulin resistance as well as in formation of hypercoagulability in diabetic patients. Altered synthesis of soluble factors affecting coagulation, fibrinolysis and vascular endothelium seemed to play a crucial role in the prothrombotic state observed in obese and diabetic patients.

4.3 Study population

Blood samples (2 heparin-tubes and 1 serum-tube, each containing 9ml) were collected during routine consultation. Patients' history, demographics and diagnostic criteria of diabetes, plasma glucose and current treatment were obtained from their clinical record.

4.3.1 Inclusion criteria

Group 1 (n=11): Patients with initially diagnosed type 2 diabetes without therapy (fasting plasma glucose ≥ 126mg/dl and/or two-hour plasma glucose ≥ 200mg/dL during an oral glucose-tolerance-test (OGTT) and/or hemoglobin A1C ≥ 6.5%)

Group 2 (n=15): Patients with diagnosed type 2 diabetes (hemoglobin A1C ≥ 6.5%) under therapy (oral antidiabetic drugs / insulin / analogues)

Group 3 (n=13): Patients with an impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (fasting plasma glucose 100mg/dl to 125mg/dl and/or two-hour plasma glucose 140mg/dl to 199mg/dl during an OGTT)

Group 4 (n=15): Insulin sensitive patients (fasting plasma glucose < 100mg/dl and/or two-hour plasma glucose < 140mg/dl during an OGTT)

4.3.2 Exclusion criteria

Patients with leukocytosis or leucopenia, chronic inflammatory diseases, acute infection, tumor or malignant neoplasm in the last 5 years, pregnancy, chronic cardiac insufficiency, peripheral arterial disease and unstable angina pectoris could not be included in the study.

4.3.3 Ethical and legal aspects

4.3.3.1 Risks

The expected risk for the healthy volunteers involved in the study was considered as minimal. Blood was drawn by standard procedure. Possible adverse effects from the venipuncture were local hematomas, redness, pain and infection.

4.3.3.2 Protection of data privacy

All data obtained from the subjects in this study were handled with care and were not passed to a third person. Every subject was given a unique code to ensure the protection of personal data sample evaluation.

4.3.3.3 Written informed consent

Written informed consent was obtained from every patient. Every participating patient was informed thoroughly about the study. Every subject was able to withdraw from the study at any time without giving reasons.

4.4 Statistical methods

All data collected in this study was evaluated statistically using SPSS (SPSS Inc., Chicago, USA) and GraphPad Prism 5 (GraphPad Software Inc., California, USA). Data are expressed as mean +/-SEM and assessed, depending on the data's distribution, using t-test, MANN-Whitney U-test, one-way analysis of variance (one-way ANOVA) or Kruskal-Wallis one-way ANOVA. For nominal or ordinal data chi-squared test was used. P-values below 0.05 were considered as statistically significant, those below 0.01 as statistically highly significant.

4.5 Methods

Serum samples of the patients were obtained, (1450g for 15min) and stored at -20°C. A differential blood count was implemented for every obtained sample. Peripheral blood mononuclear cells (PBMCs) were separated from the heparinized blood samples by Ficoll

Paque-density gradient centrifugation (800g for 15min) and incubated for 24 hours at different conditions in cell culture medium ($2,5x10^6$ cells/ml). The supernatant and the isolated RNA of the PBMCs were stored at -80°C until further processing.

4.5.1 PBMC separation

Peripheral blood mononuclear cells (PBMC) were purified by Ficoll-Paque PLUS (GE Healthcare Bio-Sciences AB, Sweden) density gradient centrifugation. Anticoagulant-treated (heparinized) blood was diluted with one third of balanced salt solution and layered carefully over 15ml of Ficoll-Paque PLUS in a centrifuge tube. After centrifugation at room temperature (800g, 15min) lymphocytes, monocytes and platelets were obtained from the buffy coat and the interface between the Ficoll-Paque PLUS and the sample layers. This material was then centrifuged twice in balanced salt solution to purify the lymphocytes (400g, 9min). The resulting cell pellet was then resuspended with CellGro serum-free medium (Cell Genix, Freiburg, Germany) and incubated for 24h at a concentration of $2,5x10^6$ cells/ml. One third of the cells were incubated in a native state, the second third was stimulated with the addition of 12µl of diluted lipopolysaccharide (LPS) to mimic an inflammatory environment. In the last third proliferation was induced by applying 12µl of diluted phytohaemagglutinin (PHA).

Serum levels of the mentioned cytokines were measured using ELISA. The role of PBMCs and their secretion pattern was evaluated by measuring the concentration in cell culture supernatant after incubating the PBMCs in medium for 24h. To examine the changes in the secretion pattern in an inflammatory environment, one third of the PBMCs was induced by LPS, a polysaccharide that can be found in gram negative bacteria and therefore simulating a state of infection. To complete the analysis under a proliferative stimulus was tested in the last third of cultured PBMCs with addition of PHA.

After 24 hours of incubation the cells were removed from the medium by centrifugation (400g, 9min). The supernatant was stored at -80°C. The cells were further processed to isolate the RNA.

4.5.2 RNA purification and cDNA synthesis

To isolate purified RNA from the cells using Trizol (life technologies, Invitrogen™ Ambion Thermo Fisher Scientific), the samples were first lysed with 1ml Trizol Reagent, according to

the protocol. After adequate mixing of the suspension, the lysed cells were stored at -80°C, to avoid degradation of the RNA.

The addition of 200µl chloroform to the sample and incubation for 5 min on ice followed. Thereafter samples were centrifuged for 20min to separate the RNA in the aqueous phase and the lower phenol-containing organic phase. The upper aqueous phase was carefully transferred to a new tube. The equal amount of propanol was added to the sample. This step was followed by the addition of 75% ethanol and centrifugation. 1ml of 100% ethanol to each tube was added followed by a further centrifugation step.. Thereafter, the supernatant was discarded and the RNA pellet was allowed to dry at normal atmosphere. Finally the RNA pellet was resuspended in 30µlH_2O and stored for further processing at -80°C.

4.5.3 Quantitative reverse transcriptase PCR (qPCR)

mRNA expression of human PBMCs was evaluated using qPCR with Light Cycler Fast Start DNA Master SYBR Green I (Roche Applied Science, Penzberg, Germany) according to the manufacturer´s instruction. In brief a PCR cycle conditions were set as follows: 10 minutes pre-incubation with 95°C, followed by 55 cycles, with each cycle including 10 seconds at 95°C. Light Cycler Software 1.5.0 (Roche Applied Science) was used for calculation of crossing points (Cp) values that characterise the cycle number at with the fluorescence signal of the sample exceeds a background fluorescence value. Two no template controls were run at each PCR plate. [167]

Total RNA was reverse-transcribed with IScript cDNA synthesis kit (BioRad, Hercules, CA, USA) according to the manufacturer's instructions. Intron-spanning primers were designed using primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). NIH nucleotide blast software was used to evaluate the specifity of primers (https://blast.ncbi.nlm.nih.gov/). The primer pairs were synthesised by Microsynth AK (Vienna, Austria). The following sequences where used: MIF forward: 5´-GTTCCTCTCCGAGCTCACC-3´ reverse: 5´-TGCTGTAGGAGCGGTTCTG-3´; B2M forward: 5′-GATGAGTATGCCTGCCGTGTG-3′ reverse: 5′-CAATCCAAATGCGGCATCT-3′. [167]

Amplification of each target gene was performed using 3µl of diluted cDNA, 5 pmole of each primer, 4µl H2O, 7.5µl Light-Cycler SYBR Green I (Roche Applied Science, Penzberg, Germany) in a final volume of 15µl in 96 well reaction plates. β-2-microglobulin (B2M) served as a house-keeping gene. [167]

4.5.4 Enzyme-linked immunosorbent assay (ELISA)

Levels of MMP-9, TIMP-1, Lipocalin, MIF, (all Duoset kits, R&D systems, Minneapolis, USA) and hsCRP following the manufacturer's instructions were quantified in the serum and supernatant of PBMCs.

An ELISA assay detects systemic antigens in human fluids. In this study we used serum and the cell culture supernatants for protein detection. Analysed protein concentrations completed our gene expression data and indicated if changes in the gene expression actually lead to secretion of proteins and possible differences in circulating serum levels between the different groups.

First of all, the capture antibody specific to the antigen of interest was sealed on the plate, by diluting according to the manufacturers' protocol, and incubating overnight at room temperature. After 24 hours and a washing step, plates were blocked with blocking buffer to avoid unspecific binding. After another washing step, samples and the predefined standard were applied on the 96 well plate. During the incubation time of 2 hours the proteins in the sample and the standard bind to the specific capture antibody. Plates were then washed and the enzyme (biotin)-linked, detection antibody was added.. To visualise the amount of bound detection antibody, a fluorescent signal must be induced. This was realised by the application of streptavidin-horse radish peroxidase (HRP). The HRP bound to the biotin, which is linked to the detection antibody. To initialise the necessary colour reaction 3,3′,5,5′-Tetramethylbenzidine (TMB) was added and created a blue colour by cleaving the streptavidin-HRP. This reaction is vulnerable to light exposition and was therefore protected from direct light. The reaction was finally stopped by the addition of 50 µl of 2% sulphuric acid. By photometric analysis with the Wallac Multilabel counter 1420 (PerkinElmer, Boston, Massachusetts, USA) the optical density was measured by a laser with a determined wavelength of 450nm. The deflection in combination with the defined standard curve defined the quantity of protein detected in the samples.

5 Results

To test our hypothesis, that PBMCs displaying an altered secretion pattern and increased levels of proinflammatory cytokines play an important role in the pathogenesis of diabetes mellitus type 2 and in the increase of cardiovascular risk, we obtained serum and heparinised blood samples from 53 volunteers.

The study population was composed of patients at different aggravation levels. From a group with impaired fasting glucose (n=13), patients with newly diagnosed diabetes mellitus type 2 without medication (n=11), a group with DM 2 under therapy (n=15), and healthy volunteers (n=15). Thus we were able to detect changes under the conditions of normal secretion, inflammation and increased proliferation.

In **Fehler! Verweisquelle konnte nicht gefunden werden.** the concentrations are quoted for very condition tested with the mean and the standard deviation. Following cytokines were measured during the study: hsCRP, MIF, MMP-9 and TIMP-1. The protein concentrations were measured in serum and supernatant at different conditions. hsCRP in the supernatant was below the detection limit of the ELISA and therefore not shown. The Table 5.1 is intended to provide an overview on the protein concentrations in the serum and supernatant of the PBMCs. The protein concentrations will be described in detail in the results section.

Cytokine	Condition	Healthy Control	IFG	Under Therapy	Initial Diagnosis
		Mean (SD)			
Serum					
hsCRP (µg/ml)		1586 (±1871)	2212 (±2157)	6301* (±3871)	3636* (±2580)
MIF (pg/ml)		1727 (±1264)	2451 (±2102)	2214 (±986)	1629 (±688)
MMP-9 (µg/ml)		450 (±346)	415 (±188)	432 (±141)	407 (±192)
TIMP-1 (ng/ml)		328* (±65)	373 (±95)	458 (±155)	402 (±101)
Supernatant					
MIF (pg/ml)	**native**	1355 (±1225)	1194 (±1034)	1327 (±920)	1283 (±590)
	LPS	1678 (±1259)	1498 (±707)	2638 (±733)	1629* (±998)
	PHA	1517 (±1193)	1194 (±831)	2003 (±1328)	1225 (±806)
MMP-9 (pg/ml)	**native**	1695 (±1472)	1560 (±1176)	1308 (±747)	1184 (±705)
	LPS	2853 (±2615)	2235 (±1984)	1786 (±709)	1731 (±1294)
	PHA	6597 (±1872)	5344 (±1714)	5111* (±2524)	4352 (±1993)
TIMP-1 (ng/ml)	**native**	9.37 (±2.91)	9.02 (±3.14)	8.88 (±4.68)	7.79 (±2.40)
	LPS	19.98 (±7.40)	26.05 (±11.64)	27.24 (±12.51)	16.46 (±5.62)
	PHA	14.16 (±5.22)	15.51 (±9.38)	18.07 (±6.85)	13.64 (±5.13)

Table 5.1 Cytokine concentrations in serum and supernatant of PBMCs
* marks values with significant difference compared to the control group and will be described in the results section, IFG=Impaired fasting glucose, SD=Standard deviation

5.1 Serum levels of hsCRP are elevated in diabetic patients

To test the cardiovascular risk of the various groups a measurement of hsCRP in the serum was measured for every participant (n=54). Significantly elevated levels were found in diabetic patients under treatment (p=0.0037), as well as in the diabetic patients after initial diagnosis, without any medication (p=0.0004) compared to the healthy volunteers. The D'Agostino and Pearson normality test was calculated and a Kruskal Wallis test and Mann Whitney test was performed. These increased values of hsCRP depict a clearly elevated cardiovascular risk in diabetic patients and further research for possible causes and additional variances in the gene expression should be conducted. HsCRP values of patients with initial

diagnosis, compared to patients with impaired fasting glucose, are increased (p=0.0012). **Fig. 5.1**

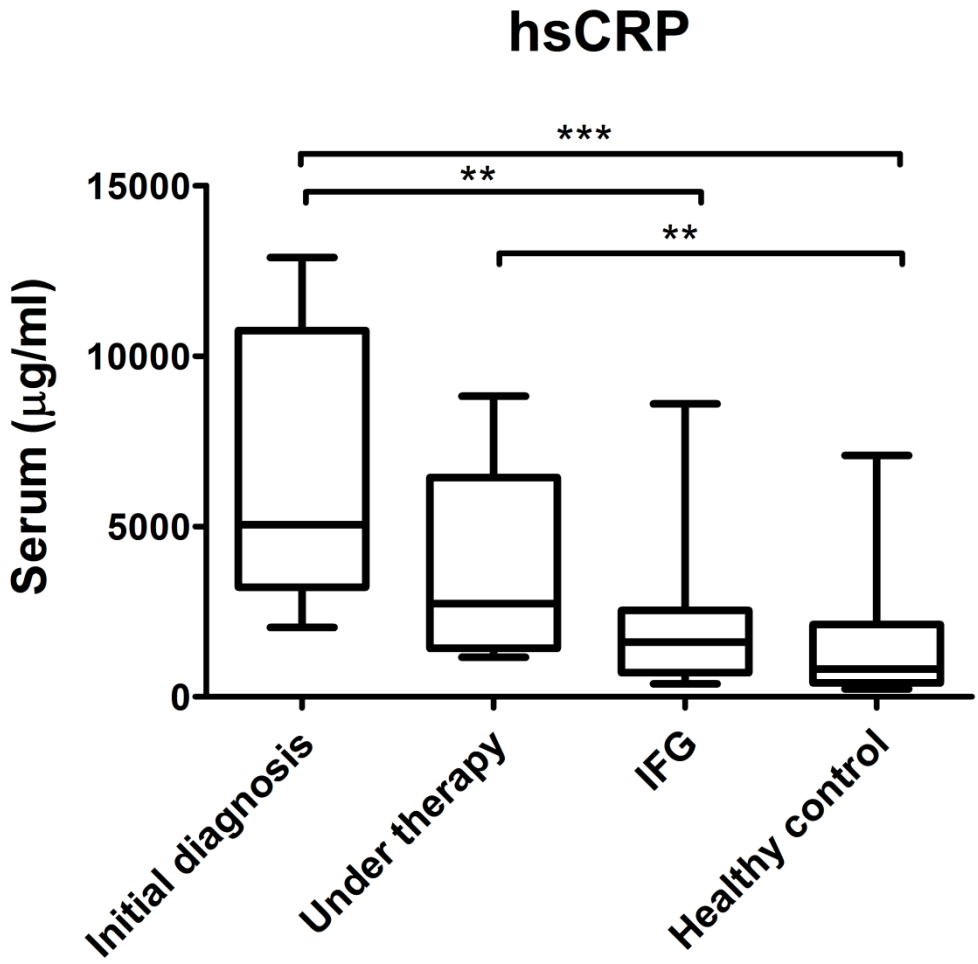

Fig. 5.1. Serum concentrations (µg/ml) of hsCRP in patients with initial diagnosis (without therapy, n=11), under treatment of diabetes mellitus type 2 (n=15), impaired fasting glucose (n=13) and healthy volunteers (n=15). IFG=Impaired fasting glucose

5.2 MIF expression is elevated PBMC of diabetic patients

To test our theory of the impact of altered MIF values on the evolvement of diabetes mellitus type 2 we performed a qPCR analysis of MIF expression in the PBMCs of the different groups under native conditions and under an inflammatory stimulus (LPS) the result was an

increased fold change in diabetic patients under therapy (mean 5.265 ± 1.460) and patients with impaired fasting glucose (mean 4.895 ± 1.366) compared to the healthy control group under LPS stimulation (mean 1.332 ± 0.4022). The number of samples per group was 4. **Fig. 5.2**

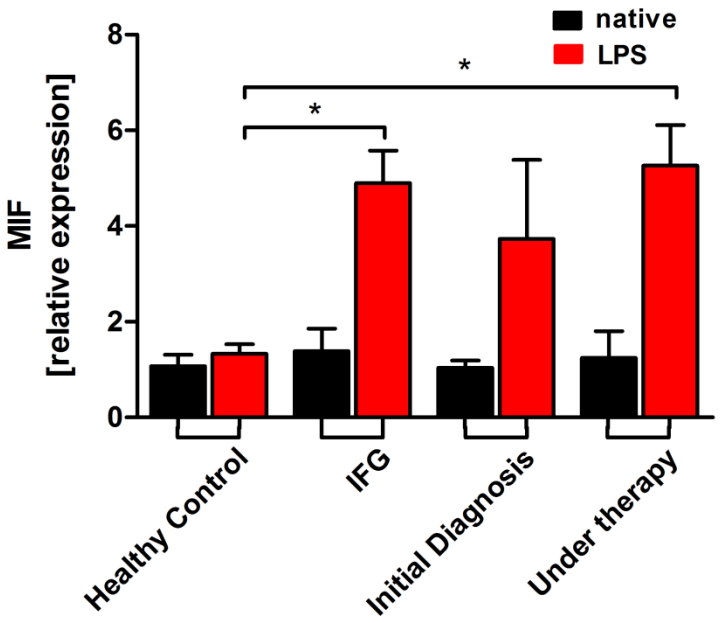

Fig. 5.2. The relative expression of MIF in the PCR in the PBMCs after 24 h of incubation without stimulation (black) and after addition of LPS (red) in patients with initial diagnosis (without therapy, n=4), under treatment of diabetes mellitus type 2 (n=4), impaired fasting glucose (n=4) compared to healthy volunteers (n=4).. IFG=Impaired fasting glucose

5.3 Protein levels of MIF in the serum and supernatant

The protein concentration was tested in the serum and in the supernatant of the PBMCs, after 24h of incubation. One third of the PBMCs were incubated without any stimulation (native), the second third was stimulated with LPS and the last third was stimulated with PHA. The cytokine concentrations are depicted in pg/ml. A significant difference was seen between the MIF concentration after LPS stimulation of cells from patients with initial diagnosis (2638 ± 732.9) versus healthy controls (1678 ± 1259) (p= 0.0170). No difference was displayed under native condition, and after induction of proliferation.

In the serum no significant changes in the protein levels (pg/ml) could be found between patients with initial diagnosis 2214 ± (986.2), diabetic patients under therapy 1629 ± (688.3) impaired fasting glucose 2451 ± (2102) and the healthy controls 1727 ± (1264).

Fig. 5.3

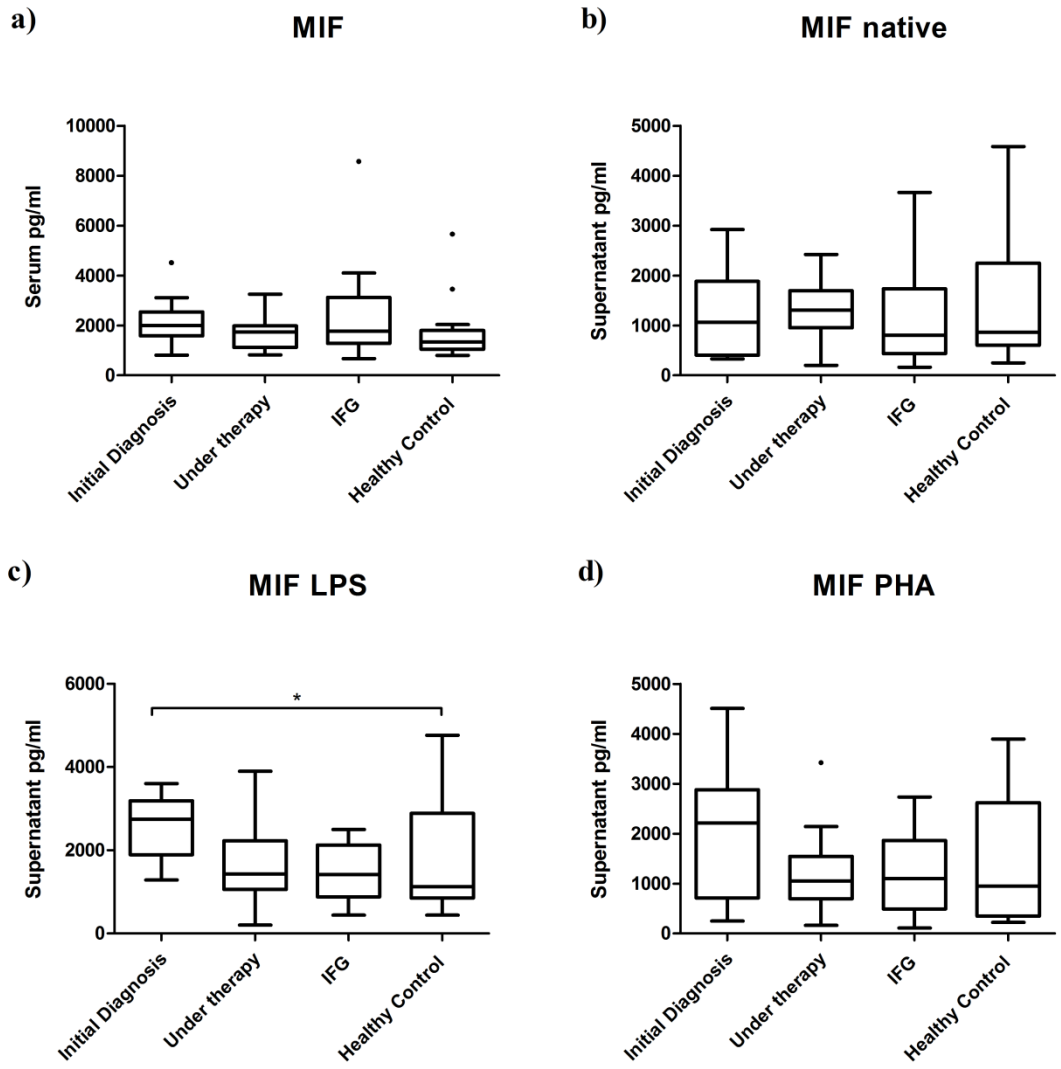

Fig. 5.3. Levels of MIF in the a) serum, b) supernatant of PBMCs after 24h of incubation, without stimulation, c) after stimulation with LPS and d) after PHA stimulation in patients with initial diagnosis (without therapy), under treatment of diabetes mellitus type 2 , impaired fasting glucose compared to healthy volunteers. IFG=Impaired fasting glucose

5.4 Protein levels of MMP-9 in the serum and supernatant

Measured protein concentrations in the serum evinced no significant distinctions between the groups. The only disparity was seen in the MMP-9 secretion between diabetic patients under therapy (4352 ± 1993) and the healthy volunteers (6597 ± 1872) after PHA stimulation. A significant reduction in the protein level of MMP-9 in the diabetic patients with treatment could be seen (p= 0.0036).

In the serum no significantly altered changes in the protein levels (µg/ml) could be found between patients with initial diagnosis 431,7 ± (140,5) , diabetic patients under therapy 407,2 ± (192,4), impaired fasting glucose 414,8 ± (188,2) and the healthy controls 449,6 ± (346,2).

Analysing the supernatant after 24h of incubation no alterations between the patients with initial diagnosis 1308 ± (746.8), DM 2 under therapy 1184 ± (704.9) and impaired fasting glucose 1560 ± (1176) compared to the healthy control group 1695 ± (1472) were found in the unstimulated supernatant (pg/ml). Stimulation with LPS displayed a similar result with no significant distinctions. **Fig. 5.4**

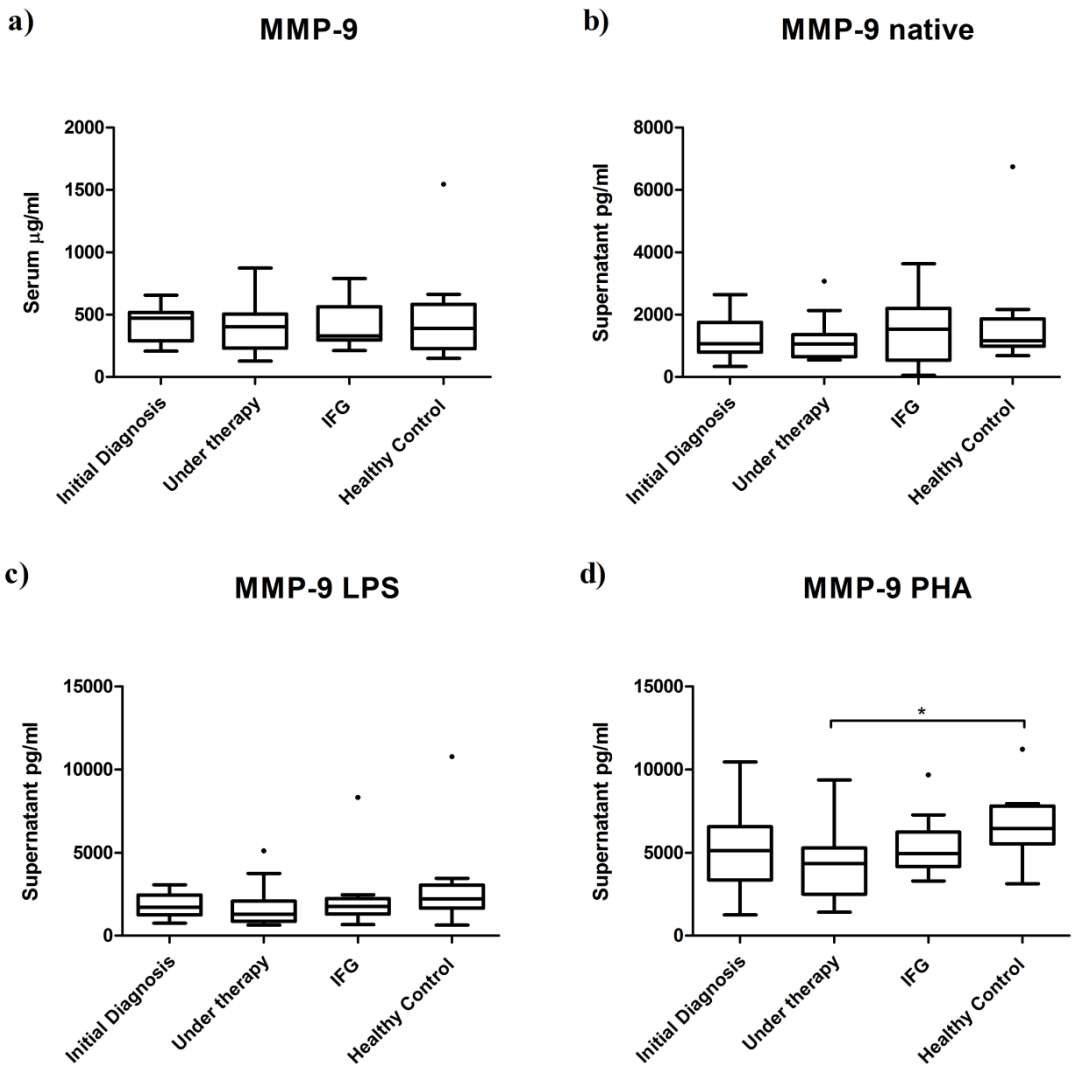

Fig. 5.4. Levels of MMP-9 in the a) serum, b) supernatant of PBMCs after 24h of incubation, without stimulation, c) after stimulation with LPS and d) after PHA stimulation in patients with initial diagnosis (without therapy), under treatment of diabetes mellitus type 2, impaired fasting glucose compared to healthy volunteers. IFG=Impaired fasting glucose

5.5 Protein levels of TIMP-1 in the serum and supernatant

Serum concentrations (ng/ml) of TIMP were significantly higher in the group of diabetic patients with initial diagnosis (457.5 ± 155) in comparison to the healthy control group (328.1 ± 64.49) (p= 0.0285).

This effect could not be seen in the supernatant of the PBMCs after 24h of incubation in native culture medium between the participants with initial diagnosis 8.883 ± (4,684), DM 2 under therapy 7,788 ± (2,395), with impaired fasting glucose 9.021 ± (3.141) and the healthy controls 9.37 ± (2.914).

After stimulation with LPS the PBMCs of the patients with initial diagnosis revealed a significantly elevated secretion level of TIMP 27.24 ± (12.51), compared to the patients under therapy 16.46 ± (5.62), (p= 0.0073). After the stimulation with PHA there was no difference between the groups. **Fig. 5.5**

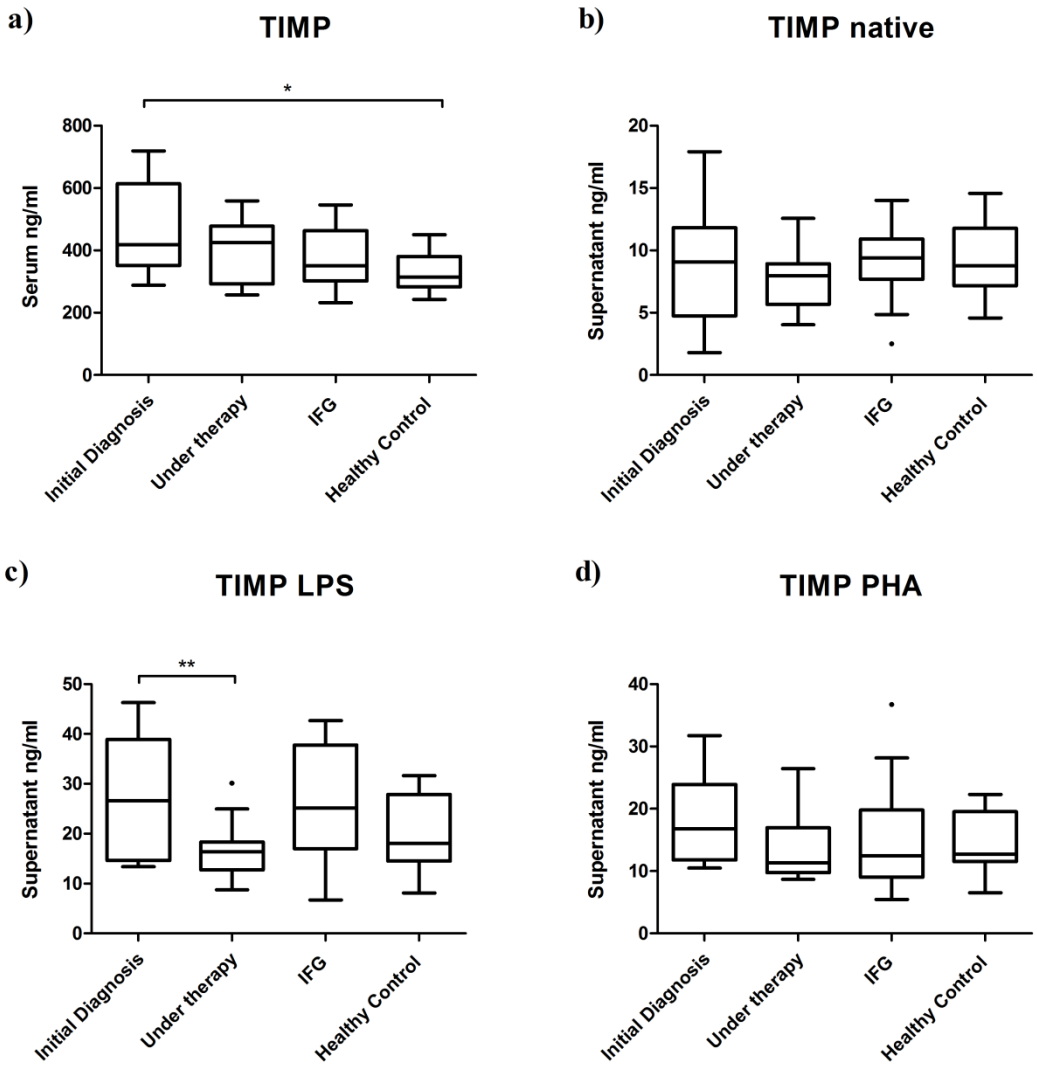

Fig. 5.5. Levels of TIMP-1 in the a) serum, b) supernatant of PBMCs after 24h of incubation, without stimulation, c) after stimulation with LPS and d) after PHA stimulation in patients with initial diagnosis (without therapy), under treatment of diabetes mellitus type 2, impaired fasting glucose compared to healthy volunteers. IFG=Impaired fasting glucose

6 Discussion

The pathogenesis of DM 2 is characterised by two principles. First of all the insulin resistance and secondly the relative lack of insulin. This impaired insulin sensitivity is induced by high levels of blood glucose, which lead to higher levels of secreted insulin. This mechanism of regulation and counter regulation creates a vicious circle, where higher doses of secreted insulin, aggravate the insulin resistance of the cells. Thus the glucose uptake of the cells is deranged and a state of hyperglycaemia is generated. This elevated circulating blood glucose values lead to further insulin secretion. [7, 33, 168, 169]

Yet the origin of diabetes is not that simple, since a high calorie uptake, a sedentary lifestyle and obesity is widely spread and not every obese patient develops a fulminate diabetes mellitus type 2. [2, 11] As previously described, in diabetes mellitus type 1 an immunological trigger plays a crucial role in the outbreak of DM 1. [33] So is the immune system also involved in the evolvement of DM 2?

A recent study revealed that MIF is an autocrine regulator of insulin secretion and enhances glykolysis in muscle cells via the enhancement of fructose 2,6-bisphosphate (F2,6BP). [170] These findings may encourage our hypothesis of its role in the vicious circle of insulin resistance resulting in DM 2. In previous studies from Herder et al. higher levels of MIF were discovered in patients with DM 2 and impaired fasting glucose. [171] Another important aspect of MIF is its contribution to the proinflammatory state in adipose tissue. [141] A case-cohort study showed no significant difference in the serum levels of MIF in diabetic males compared to healthy controls. [128] Therefore we hypothesized, that the increased MIF-level is not a constantly elevated value, but a trigger is needed to provoke the rise in circulating MIF concentrations. Due to the chronic inflammation in adipose tissue, we wanted to show, that this trigger may be an infection and would be a possible explanation, that in some studies MIF was significantly elevated in diabetic patients and in other studies not. [115, 121, 128]

To analyse the function of the cells in a native and proinflammatory state we included only patients without any acute or chronic infections, antiinflammatory medication, leukopenia or leukocytosis.

LPS, also known as endotoxins, are found in the outer membrane of gram-negative bacteria with induce a strong inflammatory immune response in humans [172] Using LPS for the stimulation of PBMC we aimed to mimic the processes of bacterial infection in humans. After the LPS stimulation the PBMCs of the patients with initial diagnosis secreted significantly higher levels of MIF, than the healthy controls. This altered secretion pattern might indicate that MIF expression is only de-regulated in diabetic patients during bacterial infection whereas under normal conditions the systemic levels of MIF were comparable between diabetic patients and healthy controls. However as diabetic patients are prone to develop bacterial infections such as chronic wounds it would be interesting to investigate whether MIF levels are elevated systemically or locally at the site of infection in these patients. [173-177] If MIF is elevated its role in orchestrating the immune response should be evaluated. Further research is necessary for verification of this hypothesis with a higher number of persons included. Yet if these results could be confirmed, new possibilities in the prevention of Diabetes mellitus type 2 with antiinflamatory medication could be conceivable.

Additionally the diabetic patients included in the study exposed a higher level of hsCRP, an inflammatory marker for cardiovascular events, important in the primary prevention. Patients with a medical history of peripheral artery disease, thrombosis, myocardial infarction or stroke were excluded from the study population. MIF had a significant impact on the development of atherosclerotic plaques and plaque instability as shown in a MIF knockout mice [148]. Therefore, elevated hsCRP levels and MIF concentrations in diabetic patients during infection might additionally increase the risk for cardiovascular complications, such as myocardial infarction or stroke. [76, 125, 146, 151-153]

Beside hsCRP and MIF we evaluated the matrix metallopeptidase-9 (MMP-9). MMP-9 was selected in this study as it is associated with the genesis and rupture of atherosclerotic plaques, acute myocardial infarction and unstable angina. [178]

Therefore we wanted to investigate whether the levels of MMP-9 are increased in an early level of diabetes in serum or in the supernatant after LPS or PHA stimulation. However, we could not detect any difference in the MMP-9 levels in the serum or in the supernatant with the solitary exception of significant reduced MMP-9 concentrations in diabetic patients under therapy in comparison to the healthy controls.

On the other hand the levels of TIMP-1 (the inhibitor of MMP-9) were significantly elevated in the serum of patients with newly diagnosed diabetes, in contrast to the healthy volunteers.

TIMP-1 for a long time was solely seen as the inhibitor of the MMPs. However, nowadays a growing body of evidence indicates that TIMP-1 exerts several immunological functions beside those of MMP inhibition [157, 179, 180] TIMP-1 is also associated with heart failure and left ventricular hypertrophy [162] and elevated levels are related to mortality risk after myocardial infarction and stroke. [180, 181] Furthermore TIMP-1 leads to a reduced degradation of the insulin-like growth factor -binding protein 3 (IGF-BP3). The resulting higher levels of IGF-BP3 reduce the amount of IGF-II and cause a decreased IGF-I receptor signalling. [182] It might be speculated that the insulin resistance is increased by this mechanism. Yet further in vitro and in vivo analysis are necessary to explore the role of TIMP-1 in the pathogenesis of diabetes.

Conclusion

Our results further substantiate previous observations that diabetic patients have higher levels of hsCRP, indicating a higher risk for cardiovascular events. In addition we were able to show that PBMC of diabetic patients in response to endotoxins secrete higher amounts of MIF, as compared to healthy controls. Additional studies assessing the role of MIF in the pathogenesis of diabetes are warranted.

7 Figure legend

8 Table Index

9 References

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10 Abbreviations

ACE	Angiotensin-converting-enzyme
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADAMs	A disintegrin and metalloproteinase
ADAMTS	Disintegrin metalloproteinases with thrombospondin motifs
ARB	Angiotensin receptor blocker
a-SMA	Alpha-Smooth muscle actin
ATP	Adenosine triphosphate
BMI	Body mass index
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CVD	Cardiovascular disease
DCCT	Diabetes control and complications trial
DM	Diabetes mellitus
DSME	Diabetes self-management education
ECG	Electrocardiogram
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay (ELISA)
F2,6BP	Fructose 2,6-bisphosphate
GADA	Glutamic acid decarboxylase
GDM	Gestational diabetes mellitus
GFR	Glomerular filtration rate
GLP-1	Glucagon like peptide-1
GLUT-1	Glucose transporter 1
GM-CSF	Granulocyte macrophage-colony stimulating factor
HbA1c	Haemoglobin A1c

HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HNF-1α	Hepatocyte nuclear transcription factor 1α
HNF-1β	Hepatocyte nuclear transcription factor 1β
HNF-4α	Hepatocyte nuclear transcription factor 4α
HRP	Horse-radish peroxidase
hsCRP	High sensitivity C-reactive protein
IA-2A	Islet antigen-2 antibody
IAA	Anti-insulin-autoantibodies
IADPSG	International Association of Diabetes and Pregnancy Study Groups
ICA	Islet cell autoantibodies
IFG	Impaired fasting glucose
IFN-γ	Interferon-γ
IGF	Insulin-like growth factor
IGF-BP3	Insulin-like growth factor -binding protein 3
IGT	Impaired glucose tolerance
IL-1	Interleukin-1
IL-6	Interleukin-6
INS1	Insulin gene 1
IPF-1	Insulin promoter factor-1
LADA	Late onset Autoimmune Diabetes in the Adult
LDL	Low-density lipoprotein
LGA	Large-for-gestational-age
LPS	Lipopolysaccharide
MAC	Anti-macrophage antibody
MCP-1	Monocyte chemoattractant protein-1
MHC	Major histocompatibility complex
MIF	Macrophage migration inhibitory factor
MMP-9	Matrix metalloproteinase-9

MODY	Maturity Onset Diabetes of the Young
NDDG	National Diabetes Data Group
NIH	National Institutes of Health
OGTT	Oral glucose tolerance test
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PHA	Phytohaemagglutinin
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Real-time polymerase chain reaction
SCID	Severe combined immunodeficiency
SMBG	Self-monitoring of blood glucose
TIMP	Tissue inhibitors of metalloproteinases
TMB	3,3′,5,5′-Tetramethylbenzidine
TNF-α	Tumor necrosis factor-α
TSH	Thyroid-stimulating hormone
UDM	Undiagnosed diabetes
UKPDS	UK Prospective Diabetes Study
VCAM-1	Vascular cell adhesion molecule-1,
WGKK	Wiener Gebietskrankenkasse
WHO	World Health Organisation
ZnT-8	Zinc transporter 8

11 Curriculum Vitae

Elisabeth Maria Simader

PERSONAL BACKGROUND

Nationality: Austria

Family Status: unmarried

Date and Place of Birth: August 24th, 1990, Vöcklabruck, Austria

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EDUCATION

1997 – 2001	Elementary School, Vöcklamarkt, Austria
2001 – 2009	Federal Austrian High School (BG Vöcklabruck) with emphasis on modern languages, Austria
2009	Matura (high school graduation)
2009 – 2010	Student at the University of Business and Administration of Vienna, WU, Austria
2010 – Present	Medical Student at the Medical University of Vienna, MUW Austria
2013 – 2015	Student Research Fellow at the Christian Doppler Laboratory for Diagnosis and Regeneration of Cardiac and Thoracic Diseases, Medical University of Vienna, Austria
2016 – Present	Student Research Assistant at the FFG-Project "Aposec", Medical University of Vienna, Austria

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CLINICAL TRAINING

2012/07	Clinical Clerkship at the **Department of Surgery**, Empress Elisabeth Hospital, Vienna, Austria (4 weeks)
2012/08	Clinical Clerkship in **Primary Care**, Dr. Maria Gremmel, Vienna, Austria (2 weeks)
2013/07	Clinical Clerkship at the **Department of Emergency Medicine**, State

	Hospital Vöcklabruck, Austria (2 weeks)
2013/08	Clinical Clerkship at the **Department of Cardiology**, Hospital of Merciful Sisters, Vienna, Austria (4 weeks)
2013/02	Student Surgery Assistant at the University Clinic of **Transplantation**, General Hospital Vienna, Medical University of Vienna, Austria
2014/08	Clinical Clerkship at the **Department of General Surgery**, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong (4 weeks)

CONTINUING EDUCATION

2013/01	Internal intensive medicine-training, Univ.-Prof. Dr.med.univ. Thomas Staudinger, Medical University of Vienna, Austria
2013/07	Summer School/Emergency Simulation Training, General Hospital Linz, Austria
2014/04	Methodenseminar: "Molekularbiologie"- Methods Seminar „Molecular Biology", Medical University of Vienna, Austria
2014/05	Methodenseminar: "Statistik" – Methods Seminar "Statistics"- Dr.rer.nat. Angelika Geroldinger, Medical University of Vienna, Austria
2014/11	Biometry II: Statistical Testing, PE-Seminar, Medical University of Vienna

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ETHICS COMMITTEE APPROVAL

"Changes in the biological function of peripheral mononuclear cells in diabetes mellitus"
EK Nr. 1510/2013, Ethics Committee Medical University of Vienna

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CONGRESSES AND MEETINGS

2014/6	55. Österreichischer Chirurgenkongress, Gesellschaft für Chirurgie, Graz Poster Presentation as representative of Dr. Matthias Zimmermann - Bone conditioned medium increases proteoglycan-4 (lubricin) in mesenchymal cells via TGF-betaR1
2014/12	4th EACTS Meeting on Cardiac and Pulmonary Regeneration and Stem Cell Technology, Bern

2015/6	Jahrestagung der ÖKG - Österreichische Kardiologische Gesellschaft, Salzburg Poster presentation in basic science - Difference in MIF production by PBMC in patients with Diabetes mellitus type II

AWARDS AND GRANTS

2014/01	Advancement Scholarship – Medical University Vienna, Austria
2014/01	Research Scholarship – Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria
2014/07	Research Scholarship – Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria
2015/01	Research Scholarship – Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria
2015/07	Research Scholarship – Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria

TEACHING ACTIVITY

2016/02 – 2016/07	Tutor at the Medical University of Vienna, University Clinic of medical Education and Training OSCE exam preparation assistant for Surgical skills
2016/02 – 2016/07	Tutor at the Medical University of Vienna, University Clinic of Anaesthesia for Dyspnoea
2015/10 – 2016/02	Tutor at the Medical University of Vienna, University Clinic of Anaesthesia for Surgical skills, Blood serology, Performance diagnostics

2015/09 – 2015/09	Tutor at the Medical University of Vienna, University Clinic of medical Education and Training Return Week exam assistant
2015/07 – 2015/07	Tutor at the Medical University of Vienna, University Clinic of medical Education and Training Return Week exam assistant
2015/02 – 2015/07	Tutor at the Medical University of Vienna, University Clinic of medical Education and Training OSCE exam preparation assistant for Surgical skills
2014/10 – 2015/02	Tutor at the Medical University of Vienna, University Clinic of Anaesthesia, Surgical skills, Blood serology, ECG, Spirometry
2013/10 – 2014/02	Tutor at the Medical University of Vienna, University Clinic of Anaesthesia, Surgical skills, Blood serology

SCIENTIFIC EXCURSIONS

2013/7	APOSEC study, Kaposvar, Hungary

CURRENT STUDIES

Diploma thesis on: Changes in the protein secretion and gene expression of peripheral blood mononuclear cells in diabetic patients

PUBLICATIONS

Analysis of the Secretome of Apoptotic Peripheral Blood Mononuclear Cells: Impact of Released Proteins and Exosomes for Tissue Regeneration.
Beer L, Zimmermann M, Mitterbauer A, Ellinger A, Gruber F, Narzt MS, Zellner M, Gyöngyösi M, Madlener S, Simader E, Gabriel C, Mildner M, Ankersmit HJ.
Scientific Reports 2015 Nov 16;5:16662. doi: 10.1038/srep16662.

METHODS

Cultivation of human cell lines

Immunohistochemistry

ELISA

PCR