Chapter 6

Summary and general discussion
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As the number of patients with diabetes mellitus and its complications is increasing worldwide, more research about pathogenesis and prevention of the disease and its complications is desperately needed. The rising number is mainly caused by individuals with type 2 diabetes. However, also an increase in individuals with type 1 diabetes has been observed. In high-income countries about 7% to 12% of all diabetic patients have type 1 diabetes and the worldwide prevalence is increasing by 3% every year. The pathophysiological mechanisms leading to vascular complications (e.g. cardiovascular disease (CVD), albuminuria and retinopathy) have not been fully elucidated.

To date, altered extracellular matrix (ECM) remodeling has been extensively investigated in animal models of type 1 diabetes. In contrast, studies investigating associations between altered ECM remodeling in humans with type 1 diabetes are scarce, inconsistent and have various limitations, like small study groups and minimal adjustments for potential confounders. The lack of robust studies in humans has stimulated us to investigate the role of ECM remodeling in vascular complications. In this thesis we studied patients with type 1 diabetes included in three large study cohorts (EURODIAB, LEACE and PROFIL). Serum and plasma samples were used to evaluate circulating levels of markers of the matrix metalloproteinase (MMP) - tissue inhibitor of metalloproteinase (TIMP) system and associations with complications were investigated using the original data provided. In this section, the key findings of the four studies are summarized and discussed in light of the current knowledge of ECM remodeling by MMPs and TIMP-1, enzymes fundamentally involved in both vascular collagen and elastin proteolysis, in type 1 diabetes.

MMPs, TIMP-1 and vascular complications

In chapter two, a positive association is described between serum levels of TIMP-1 and prior CVD in the cross-sectional nested case-control EURODIAB study including 493 type 1 diabetic individuals. Although the association between circulating TIMP-1 and prior CVD was observed in two studies with individuals with type 2 diabetes and a history of coronary artery disease, we were the first who published the association of TIMP-1 with prior CVD in type 1 diabetes. Higher levels of TIMP-1 may reflect arterial remodeling after myocardial infarction, but can also be a marker of (post-myocardial infarction) cardiac fibrosis, potentially combined with decreased left ventricular function.

Unfortunately, we do not have data on left ventricular function to evaluate whether or not higher serum TIMP-1 levels are also attributable to altered ventricular function.

In addition, we showed that serum levels of MMP-2, -3, -10 and TIMP-1 were positively associated with macroalbuminuria, whereas only MMP-2 was positively associated with microalbuminuria. Similar cross-sectional analyses in the prospective LEACE study (n=370) support these results. MMP-2 and TIMP-1 were also associated with macroalbuminuria after full adjustment for potential confounders (unpublished data).
Moreover, analyses in the cross-sectional PROFIL study (n=638) showed similar associations between MMPs and TIMP-1 and macroalbuminuria as in the EURODIAB study (unpublished data). Variation of these results may be explicable by a smaller number of patients in the LEACE study, differences in presence of vascular complications between the studies and by the fact that in EURODIAB and PROFIL serum samples and in LEACE plasma samples were used for the measurements. Differences between serum and plasma measurements of MMPs and TIMP-1 will be discussed in the methodological considerations section. Our findings are supported by previous studies that also observed associations between MMP-2,\(^{2,29}\) MMP-3,\(^{30}\) MMP-10\(^{20}\) and TIMP-1\(^{1,11}\) and albuminuria in animal models of diabetes and in humans. These results indicate that these MMPs and TIMP-1 may provide therapeutical targets to prevent (deterioration of) diabetic kidney disease.

In chapter two, we also showed the cross-sectional association between circulating MMP-2 and proliferative retinopathy. MMP-2 is overall one of the most studied MMPs in diabetic retinopathy and other studies also observed clear associations between MMP-2 and retinopathy.\(^{4,15,17,24}\) MMP-2 is one of the main MMPs that degrade type IV collagen, which is present in glomerular and retinal basement membranes, and tight junction proteins. Higher MMP-2 activity can lead to increased remodeling and increased permeability\(^{5,15,17}\) in both tissues. In addition to albuminuria, MMP-2 might provide a target to prevent proliferative retinopathy.

Although circulating and tissue levels of MMP-9 have been associated with both diabetic nephropathy\(^{8,9,12,31}\) and retinopathy,\(^{15,18,24}\) we did not observe these associations in all three studies. This discrepancy might be caused by different stages of disease. In addition, MMP-9 may be produced mainly at the initial phase of ECM remodeling after which its significant role diminishes during the process of remodeling and is taken over by other MMPs.

**MMPs, TIMP-1 and incident CVD and all-cause mortality**

In the third chapter, we showed that higher levels of MMP-2 at baseline were associated with incident CVD during a median follow-up of 12.3 years. In addition, higher baseline levels MMP-1, -2 and -3 were associated with increased all-cause mortality. Evidence regarding associations between markers of ECM remodeling and incident CVD in individuals with type 1 diabetes is not available, but results of several studies in non-diabetic individuals are in line with our finding.\(^{32-38}\) Mechanistically, MMP-2 is associated with plaque rupture, thrombus formation and vasoconstriction.\(^{33,37}\) Moreover, MMP-2 can lead to an increase of vascular fibrosis, decrease of elastin content and increase of vascular smooth muscle cell migration and may as such contribute to pathological vascular remodeling.\(^{32,38}\) A mechanism, although speculative, explaining our finding regarding levels of MMP-1 and all-cause mortality in individuals with type 1 diabetes is the degradation of collagen type I, II, III, VII, X and other matrix proteins. In addition, MMP-1 is associated with altered vascular remodeling, probably contributing to
aneurysmatic vascular disease, as discussed in chapter 4. As MMP-3 has mainly the same substrates as MMP-2, we hypothesize that MMP-2 and MMP-3 contribute to remodeling in disease states through a similar mechanism.

Mediation through estimated glomerular filtration rate

Additional analyses showed that the associations between MMP-2 and incident CVD and between MMP-3 and all-cause mortality were attenuated after adjustment for estimated glomerular filtration rate (eGFR). We also showed that MMP-3, but not MMP-2, was significantly associated with decrease in eGFR during follow-up. The non-significant association between MMP-2 and decrease in eGFR might be due to the relatively small number of patients (n=317). In our opinion there are two possible explanations for the eGFR-mediated associations between MMPs and incident CVD/all-cause mortality. Firstly, decreased eGFR might cause accumulation of circulating MMP-2 or MMP-3, contributing to incident CVD or all-cause mortality, respectively. Secondly, higher levels of MMP-2 or MMP-3 could contribute to decreased eGFR, which is a known risk factor for incident CVD and mortality. Unfortunately, the design of our study did not allow us to differentiate which pathway could have caused our findings.

Low-grade inflammation and endothelial dysfunction

In the studies presented in chapter two and three, the associations of MMPs with vascular complications were adjusted for markers of low-grade inflammation (LGI) and endothelial dysfunction (ED). We calculated z-scores for LGI and ED based on a panel of markers reflecting LGI and ED. Levels of MMPs are influenced by the inflammatory environment and may even be considered as markers of inflammation. However, we showed that our associations of MMPs with vascular complications remained after adjustment for markers of LGI and ED. Although we believe that we made well-reasoned scores of LGI and ED, we cannot exclude, however, that other markers of LGI and ED may influence our results and therefore we have to interpret our results with care.

MMPs, TIMP-1 and markers of arterial stiffness

In chapter four, we showed that MMP-1 was inversely associated with office pulse pressure (PP), whereas MMP-2 was positively associated with office PP. In addition, MMP-2 was also positively associated with brachial and central 24-h PP. Based on the structure of the thoracic aorta, it might be that MMP-1 degrades collagen type 1 in the thoracic aorta causing a decrease in collagen content of the vessel wall, leading to increased elasticity, decreased PP, and eventually to thoracic aortic aneurysm or dissection. In contrast, MMP-2 is known to degrade type IV collagen, present in the vascular basement membrane, and elastin. In addition, both MMP-2 and MMP-3 are able to increase matrix production by cleavage of tissue transforming growth factor-β
(TGF-β) and therefore stimulate a TGF-β-induced matrix production,\textsuperscript{43,44} mainly collagen and fibronectin. Based on this mechanism we expected that MMP-2 would also be associated with higher arterial stiffness, as observed with PP. Due to the amplification phenomenon, brachial PP may not truly represent central PP in young and healthy individuals.\textsuperscript{45} However, the accelerated arterial aging in type 1 diabetes has been previously proven.\textsuperscript{46} In addition, in a large study with type 1 diabetic individuals with a mean age of 39 years, brachial PP was positively associated with incident CVD during a median follow-up of 5.3 years.\textsuperscript{47} Therefore PP may be an adequate measurement for arterial stiffness at younger age in type 1 diabetic individuals.

In contrast to previous studies without individuals with type 1 diabetes,\textsuperscript{48-50} circulating MMP-2 was not associated with carotid-femoral pulse wave velocity (cfPWV). In contrast, circulating levels of MMP-3 were associated with cfPWV. In additional analysis we found that MMP-3 was associated with PP in individuals above the age of 40. When we further analyzed the data we concluded that adjustment for both age and duration of diabetes may lead to overadjustment. Reanalysis of the data with only age as a potential confounder revealed a significant positive association between MMP-2 and cfPWV and the association between MMP-3 and cfPWV became even stronger. The intrinsic characteristics of MMP-2 and MMP-3, both largely sharing the same substrates and effect on matrix production, combined with the current literature give us plausible explanations for our findings. In addition, the difference in composition between thoracic and abdominal aorta could also indicate that MMP-3 is able to alter abdominal aortic tissue and to increase cfPWV. MMP-1 showed a borderline inverse association with cfPWV, which is largely in line with the finding between MMP-1 and PP.

Advanced glycation endproducts and the MMP-TIMP system

In the fifth chapter, we investigated associations between serum levels of advanced glycation endproducts (AGEs) and markers of the MMP-TIMP system. AGEs are involved in CVD in both type 1\textsuperscript{51} and type 2 diabetes.\textsuperscript{52} Literature provides lots of evidence that AGEs are able to induce MMP production.\textsuperscript{29,53-66} In vitro studies have shown AGE-induced MMP expression in various cells that are associated with vascular complications.\textsuperscript{29,57,62,65} We studied associations between circulating levels of protein-bound N\textsuperscript{ε}-(carboxymethyl)lysine (CML), N\textsuperscript{ε}-(carboxyethyl)lysine (CEL), S-hydroxy-S-methylimidazolone (MG-H1), pentosidine and MMP-1, -2, -3, -9, and -10, and TIMP-1. We showed that CML, an extracellular AGE and a major ligand of the receptor of AGE (RAGE), was positively associated with circulating MMP-2 and inversely with MMP-9 after adjustment for potential confounders. CEL was positively associated with MMP-3 and TIMP-1 and MG-H1, together with CEL representing intracellular methylglyoxal-derived AGEs, was only associated with TIMP-1. Circulating pentosidine, a cross-linking AGE, was not associated with circulating MMPs or TIMP-1.

Thus, our study showed several independent associations of several specific AGEs with specific MMPs or TIMP-1. Our findings indicate that CML is associated with increased
matrix turnover, as indicated by the association with MMP-2. CML- and other AGE-induced MMP-2 expression have been published previously (chapter five). In contrast to CML, the intracellular AGEs, CEL and MG-H1, might decrease matrix turnover, as shown by the associations with TIMP-1. As expected, the cross-link pentosidine showed no association with MMPs or TIMP-1.

Whereas literature largely showed positive associations between AGEs and MMPs/TIMP-1, several explanations may clarify our findings of just a few associations between AGEs and markers of the MMP-TIMP system. Firstly, the contribution of AGEs to MMP-mediated ECM remodeling may be overestimated despite current literature regarding AGE-mediated MMP and TIMP-1 production. Although various AGE stimulated cells have shown to induce MMP production, most of the experiments may have been performed with a concentration of AGEs higher than under physiological conditions. Therefore, we do not know if AGE stimulation at tissue level induces similar MMP production as compared to in vitro studies. In addition, at tissue level other factors, like e.g. growth factors and cytokines, may also alter MMP production. Finally, circulating markers of both MMPs and AGEs may not truly reflect the situation at tissue level and may underestimate the observed associations.

Methodological considerations

Study populations

This thesis comprises data of individuals with type 1 diabetes derived from three large studies: EURODIAB, LEACE and PROFIL. Patient characteristics of the three studies are shown in Supplemental Table S4.1.

From the EURODIAB study, we used the follow-up cross-sectional nested case-control study, which is a subset of 543 patients, re-evaluated seven years after initial inclusion. Patients with the largest vascular burden (n=348) were compared to those without any vascular complication (n=195). In the prospective LEACE study 391 type 1 diabetic individuals were included of whom 199 had diabetic nephropathy, defined as macroalbuminuria with a urinary albumin excretion (UAE) of >300mg/24h. The other 192 individuals were normoalbuminuric (UAE <30mg/24h). The median follow-up was 12.3 years and included data on incident CVD and all-cause mortality. Prospective analyses were performed in 337 individuals, whereas data regarding arterial stiffness was present in 370 subjects. The PROFIL study included 676 individuals with type 1 diabetes and 51 non-diabetic controls. The controls were included to investigate differences between them and patients with a short duration of diabetes. In the diabetes group, 316 patients were normoalbuminuric, 169 microalbuminuric and 191 had macroalbuminuria. So albuminuric patients were overrepresented by design.
In our analyses, only patients with type 1 diabetes were included. The question remains to what extent the MMP-TIMP system acts similarly in the various types of diabetes and non-diabetic subjects. A large number of potential confounders (e.g. age, sex, duration of diabetes, glycated hemoglobin A1c, eGFR, blood pressure, lipid profiles, body mass index, smoking status, antihypertensive medication and presence of vascular complications) were present in all studies and therefore we were able to adjust our associations for these confounders. However, we cannot rule out that other factors (e.g. growth factors) influenced our results. Finally, results from the EURODIAB and PROFIL study are only based on cross-sectional analysis and therefore we can only speculate on causality in these studies.

Markers of the MMP-TIMP system

In all studies we determined the same markers of the MMP-TIMP system: MMP-1, MMP-2, MMP-3, MMP-9, MMP-10 and TIMP-1. We chose these markers based on the intrinsic characteristics of these enzymes that could contribute to vascular complications, as supported by current literature. For example, MMP-1 degrades collagen type 1,67 which is present in e.g. the aorta, and MMP-2, -3, -9 and -10 are known to degrade type IV collagen (as present in the basement membranes), fibronectin and elastin.68 So far, most studies investigated only one or two markers of the MMP-TIMP system in type 1 diabetes. Furthermore, the few studies available on human subjects show several methodological shortcomings, like small sample size or incomplete adjustments for confounders.19-22 In our study, we had the ability to study a large panel of markers of the MMP-TIMP system in a large population of individuals with type 1 diabetes with a variety of vascular complications (CVD, retinopathy, nephropathy and arterial stiffness).

The absolute values of the various MMPs and TIMP-1 differ between the three studies, as shown in chapter four. The absolute concentrations of circulating levels in LEACE differ significantly from those in EURODIAB and PROFIL, which could be explained by the fact that analyses were performed in plasma samples in LEACE, whereas serum samples were used in the other studies. Indeed, higher levels of MMP-9 and TIMP-1 were observed in serum compared to plasma levels of the same individuals.68,69 In chapter four we used cohort-specific z-scores to overcome the absolute differences in concentrations of MMPs and TIMP-1 between plasma and serum levels and we made an additional adjustment for cohort.

Several other issues should be addressed:

As discussed in the first part of this discussion, we have shown various associations between MMPs and TIMP-1 and vascular complications. Surprisingly, MMP-9, one of the most studied MMPs, was not associated with vascular complications in our studies and this in contrast to several other studies, as mentioned in chapter two, three and four. Several explanations may be applicable to this finding. MMP-9 may be one of the first MMPs to be produced, inducing biochemical and/or structural alteration leading to
vascular complications. In non-published data, we have seen in normoalbuminuric type 1 diabetic individuals that MMP-9 is associated with incident microalbuminuria after adjustment for potential confounders (LEACE study, n=31). This is in line with data published on type 2 diabetic individuals. It might be that MMP-9 precedes the production of other MMPs, after which MMP-9 levels decline. This may explain why we did not find associations between MMP-9 and established vascular complications in our subjects.

Samples from the EURODIAB and LEACE study were stored at -80 degrees Celsius and were approximately 16 years and 20 years old at the moment of analysis of our markers, respectively. It has been shown that plasma MMP-9 levels decrease even when stored at -80 degrees Celsius, while MMP-2 and TIMP-1 levels were not affected and we can not exclude the possibility that this may partly explain our observed lack of associations between MMP-9 and vascular complications. In addition, degradation after freeze-thaw cycles can influence the results too. MMP-2 levels are not influenced by repeated freeze-thaw cycles. However, pro-MMP-9 activity significantly decreases after 7 cycles, whereas MMP-9 activity does not decrease significantly. Because our samples have never been through more than five freeze-thaw cycles, we can exclude the possibility that degradation by freeze-thaw cycles has influenced our results.

Serum and plasma samples were used to measure MMPs and TIMP-1 as markers of ECM remodeling. Circulating leukocytes and platelets also produce MMPs and TIMPs and this may explain the differences observed between serum and plasma samples.

Another important issue is that we do not know if circulating levels of the biomarkers really represent tissue levels. Therefore, we cannot state with certainty that the measured markers solely reflect altered remodeling. Our results, however, consistently show that largely the same MMPs and TIMP-1 are associated with markers of renal function (eGFR and UAE) in all three studies. Our theory on the roles of MMPs and TIMP-1 in vascular complications, supported by the evidence that MMP-1, -2, -3, and -10 and TIMP-1 are associated with vascular complications in type 1 diabetes (chapter two, three and four), is also in line with current literature in animal and human studies.

The above-mentioned points provide enough arguments that the previously mentioned concerns are not major issues and thus validate our results.

Advanced glycation endproducts

We also determined serum levels of several pre-specified AGEs. AGEs are a heterogeneous group of glucose-modified proteins and AGE-induced MMP expression has been published previously. We determined serum levels of CML, CEL, MG-H1 and pentosidine in the PROFIL study and investigated associations between these AGEs and the markers of the MMP-TIMP system.

In addition to the remarks regarding the markers of the MMP-TIMP system, it might be that the circulating levels do not truly reflect tissue levels of AGEs. The AGEs represent markers of (cross-linking) extra- and intracellular AGEs and the question rises whether
or not these markers can easily translocate to the circulation. It might be that this underestimates our findings and a careful interpretation of these results is needed. To elucidate possible associations between AGEs and markers of the MMP-TIMP system further studies are needed.

Future perspectives

In this thesis we have shown cross-sectional and prospective associations between circulating markers of the MMP-TIMP system and vascular complications in individuals with type 1 diabetes. These results indicate that altered ECM remodeling is a key feature in vascular complications in individuals with type 1 diabetes. Since we have measured only a selection of markers of the family of MMPs and TIMPs, additional studies are needed to further elucidate whether or not other MMPs and/or TIMPs are associated with vascular complications. Moreover, influences of TGF-β and other growth hormones should be evaluated, as both matrix degradation/turnover, but also matrix production/accumulation are associated with vascular complications. Several studies have demonstrated that pathological remodeling can be attenuated if current treatment options are provided or intensified. Intensive glucose treatment and blockade the renin-angiotensin-aldosterone system significantly contribute to restoring the balance between MMPs and TIMPs and (partly) attenuate pathological ECM remodeling. In addition, broad-spectrum and specific MMP-inhibitors have been developed to prevent excess MMP-mediated ECM remodeling. Moreover, also antibodies against membrane-type MMPs, which are involved in the activation of MMPs, are available. These antibodies prevent MMP transformation from the inactive to the active form. Statins, bisphosphonates, thiazolidinediones, aspirin and other COX-inhibitors have shown to suppress MMP-production through anti-inflammatory mechanisms. Tetracyclines also alter MMP-activity. Further studies are needed to investigate the potential use of these different treatment strategies in order to prevent MMP-mediated ECM remodeling in clinical practice. Some of these medicines are already frequently used, so studies on their effects can be obtained in clinical studies. Furthermore, we question whether development of specific MMP-inhibiting agents will be a panacea in preventing vascular complications.

In conclusion, this thesis highlights associations between markers of the MMP-TIMP system and vascular complications in individuals with type 1 diabetes. These cross-sectional and prospective associations show that certain markers of this system may be suitable as therapeutical targets to treat or prevent vascular injury in patients with type 1 diabetes.
References

Chapter 6


Summary and general discussion

52. Hanssen NM, Beulens JW, van Dieren S, Scheijen JL, van der AD, Spijkerman AM, van der Schouw YT, Stehouwer CD, Schalkwijk CG: Plasma advanced glycation end products are associated with incident cardiovascular events in individuals with type 2 diabetes: a case-cohort study with a median follow-up of 10 years (EPIC-NL). Diabetes 2015;64:257-265


Nederlandse samenvatting
Nederlandse samenvatting

Diabetes mellitus, ook wel suikerziekte genoemd, is wereldwijd een toenemend probleem. In 2015 zijn er 415 miljoen diabetespatiënten in de wereld en dit aantal zal toenemen tot 642 miljoen in 2040. De hogere glucosewaarden in het bloed, die het kenmerk zijn van diabetes mellitus, kunnen op diverse manieren ontstaan. Bij type 1 diabetes mellitus, ongeveer 5 tot 10% van alle gevallen, ontstaat dit door afbraak van de insuline-producerende cellen in de alvleesklier, waardoor er een absoluut tekort aan insuline ontstaat. Bij type 2 diabetes mellitus is er sprake van verminderde insuline-gevoeligheid op weefselniveau en zodoende is er sprake van een relatief insuline-tekort. In dit proefschrift hebben we ons gericht op patiënten met diabetes mellitus type 1. Aangezien diabetes mellitus vaker voor komt, komt ook een toename van de complicaties van de ziekte voor en voorbeelden hiervan zijn onder andere (o.a.) problemen met de bloedvaten (vasculaire complicaties).

Hieronder verstaan we:
1. problemen van de grotere bloedvaten die kunnen leiden tot een hartinfarct en/of cerebrovasculair accident (CVA)(macrovasculaire complicaties)
2. problemen van de kleinere bloedvaten in o.a. de ogen (retinopathie) en de nieren (nepropathie) (microvasculaire complicaties).

We weten ook dat het stijver zijn van de grootste slagader in het menselijk lichaam (de aorta) een risicofactor vormt op het krijgen van vasculaire complicaties. Het optreden van deze complicaties kan leiden tot een verslechterde kwaliteit van leven en vroegtijdige sterfte. Het mechanisme dat leidt tot deze complicaties is nog niet geheel opgehelderd en aanpassingen in de samenstelling van een bloedvatwand is een van de problematische veranderingen die kan ontstaan. Echter is dit slechts onderzocht in met name dierenstudies en in enkele mensenstudies met patiënten met diabetes mellitus type 1.

Matrix metalloproteinasen (MMP’s) zijn enzymen die betrokken zijn bij het natuurlijke herstel en aanpassingen van de extracellulaire matrix (ECM), o.a. de samenstelling van de bloedvatwand. Deze ECM zorgt voor ondersteuning tussen diverse cellen in een weefsel, maar ook tussen de cellen en het omliggende weefsel. De activiteit van deze MMP’s is gereguleerd door o.a. natuurlijke remmers, tissue inhibitors of metalloproteinase (TIMP’s). De activiteit van de MMP’s kan ook worden verminderd door o.a. alfa-2-macroglobuline, bisfosfonaten, simvastatine en tetracyclines. Van de MMP’s zijn er 24 bekend en daarnaast zijn er 4 TIMP’s. Ontregeling van dit MMP-TIMP systeem kan leiden tot een veranderde samenstelling van de vaatwand en zodoende tot vasculaire complicaties.

In dit proefschrift hebben we gekeken naar associaties tussen circulerende waarden van MMP-1, -2, -3, -9 en -10 en TIMP-1 en vasculaire complicaties in patiënten met diabetes mellitus type 1. Daarnaast hebben we associaties onderzocht tussen circulerende waarden van advanced glycation endproducts (AGE’s, versuikerde eiwitten die ontstaan
ten gevolge van blootstelling van eiwitten aan glucose) en circulerende MMP’s. Er is namelijk aangetoond dat AGE’s de MMP- en TIMP-productie kunnen beïnvloeden en zodoende kunnen AGE’s bijdragen aan het ontstaan van veranderingen in de ECM.

**MMP’s, TIMP-1 en vasculaire complicaties**

In hoofdstuk twee hebben we cross-sectionele associaties onderzocht tussen circulerende waarden van eerdergenoemde markers van ECM remodelering in 493 patiënten met diabetes mellitus type 1 met en zonder doorgemaakte hart- en vaatziekten (HVZ). We hebben laten zien dat mensen met doorgemaakt HVZ hogere spiegels van TIMP-1 hadden. Er waren geen associaties tussen HVZ en circulerende MMP’s. We zijn de eersten die deze associatie hebben beschreven in patiënten met diabetes mellitus type 1, maar deze associatie is wel eerder beschreven bij patiënten met diabetes mellitus type 2.

Naast macrovasculaire complicaties hebben we ook gekeken naar microvasculaire complicaties (nier- en oogproblemen) in deze patiëntengroep. Eerst hebben we gekeken naar eiwituitscheiding in de urine (albuminurie), wat wordt gezien als teken van nierschade. We hebben aangetoond dat patiënten met macroalbuminurie (albumine-uitscheiding in de urine >300mg/24 uur) significant hogere concentraties van MMP-2, -3, -10 en TIMP-1 hadden vergeleken met patiënten met een normale albumine-uitscheiding in de urine (<30mg/24 uur, normoalbuminurie). Patiënten met microalbuminurie (albumine-uitscheiding in de urine tussen de 30 en 300 mg per 24 uur) hadden alleen significante hogere concentraties van MMP-2 vergeleken met patiënten met normoalbuminurie. Concentraties van MMP-2, -3, -10 en TIMP-1 toonden significante trends over de diverse gradaties van albuminurie: normo-, micro- en macroalbuminurie. Vergelijkbare associaties hebben we ook gevonden bij analyses in andere groepen met patiënten met diabetes mellitus type 1.

We hebben ook een positieve associatie gevonden tussen MMP-2 en proliferatieve retinopathie, een gradatie van problemen van de bloedvaten in de ogen. Dit betreft ernstige afwijkingen aan de bloedvaten van de ogen en de non-proliferatieve retinopathie is een minder ernstige variant hiervan, welke ook wel degelijk problemen kan veroorzaken. Geen van de markers was significant verschillend tussen individuen met non-proliferatieve retinopathie en individuen zonder retinopathie. De associatie tussen MMP-2 en proliferatieve retinopathie is vaker onderzocht en geobserveerd bij individuen met diabetes mellitus type 1, maar niet in een studie van deze grootte.

**MMP’s, TIMP-1 en incidente HVZ en dood**

In hoofdstuk drie hebben we associaties onderzocht tussen circulerende MMP’s en TIMP-1 en (niet-)fatale incidente HVZ en dood ongeacht de oorzaak. Hiervoor hebben we gebruik gemaakt van data van een Deense studie met 337 individuen met diabetes mellitus type 1 (LEACE) met normoalbuminurie (n=167) of macroalbuminurie (n=170)
met een mediane follow-up van 12,3 jaar. Incidente HVZ waren geassocieerd met een hogere circulerende concentratie van MMP-2 op baseline. Hogere baseline concentraties van MMP-1, -2 en -3 waren significant geassocieerd met dood, ongeacht de oorzaak, gedurende de follow-up van ruim 12 jaar. De significante associatie tussen MMP-2 en incidente HVZ evenals de associaties tussen MMP-3 en dood verdween na correctie voor nierfunctie. Daarbij waren baseline MMP-3 concentraties significant geassocieerd met vermindering van de nierfunctie gedurende follow-up, maar concentraties van MMP-2 op baseline waren dat niet. Dit geeft aan dat de relatie tussen MMP-2/-3 en de uitkomsten beïnvloed wordt door nierfunctie, maar hoe dit precies verloopt kunnen we hier niet uit herleiden.

Pathofysiologisch is MMP-2 geassocieerd met plaqueruptuur, trombusformatie en vasconstrictie. Daarbij is MMP-2 geassocieerd met toename van vasculaire fibrose en vasculaire gladde spiercelmigratie, maar ook met afname van elastine, een elastische component, in de vaatwand. Hierdoor worden de bloedvaten stijver en tevens minder elastisch. Door deze veranderingen kunnen we de associaties tussen MMP-2 en de hiervoor genoemde uitkomstmaten verklaren. De mogelijke manieren waarop MMP-1 en MMP-3 kunnen bijdragen zijn minder duidelijk.

**MMP’s, TIMP-1 en arteriële stijfheid**

Toegenomen arteriële stijfheid, het stijver worden van de bloedvaten, is een risicofactor voor het krijgen van HVZ in patiënten met diabetes mellitus type 1. In hoofdstuk vier hebben we gekeken naar cross-sectionele associaties tussen circulerende MMP’s en TIMP-1 en twee maten van arteriële stijfheid, te weten: carotis-femoralis pulse wave velocity (cfPWV) en polsdruk. Met cfPWV bepalen we de snelheid van het bloed tussen een bloedvat naar de hersenen (arteria carotis) en een bloedvat in de lies (arteria femoralis). Hoe sneller het bloed zich van het ene naar andere punt verplaatst, hoe stijver het bloedvat. Polsdruk, gedefinieerd als systolische bloeddruk minus diastolisch bloeddruk, is een andere maat voor arteriële stijfheid.

Allereerst hebben we aangetoond dat MMP-3 positief geassocieerd is met cfPWV (n=614), de huidige gouden standaard voor het meten van arteriële stijfheid. De andere markers waren niet significant geassocieerd met cfPWV. We hebben polsdrukdata van drie verschillende studies met patiënten met diabetes mellitus type 1 en de MMP/TIMP-1-bepalingen (n=1517). MMP-1 was invers geassocieerd met polsdruk en MMP-2 was positief geassocieerd met polsdruk. MMP-1 is eerder geassocieerd met verandering in de thoracale aorta leidend tot uitzetting en eventuele aneurysmavorming, welke kan leiden tot een daling van de polsdruk. MMP-2 en MMP-3 waren positief geassocieerd met maten van arteriële stijfheid en dit kan worden verklaard door eerdergenoemde pathofysiologische veranderingen door MMP-2, waarbij MMP-3 grotendeels hetzelfde effect heeft, alsmede het kunnen vrijprepareren van weefselgebonden transforming growth factor-beta.
(TGF-β) door zowel MMP-2 als MMP-3. Dit alles kan leiden tot toename van matrix productie en zodoende vasculaire stijfheid.

Advanced glycation endproducts en het MMP-TIMP systeem

De vraag blijft wat de primaire factoren zijn die kunnen leiden tot veranderingen in het MMP-TIMP systeem in individuen met diabetes mellitus type 1. In de algemene introductie staat figuur 1.2, waarin bepaalde bekende pathways naar veranderde ECM worden weergegeven. In de voorgaande hoofdstukken hebben we niet naar losse factoren gekeken die de expressie van MMP’s danwel TIMP-1 beïnvloeden. In hoofdstuk vijf hebben we gekeken naar associaties tussen circulerende AGE’s en de eerdergenoemde markers van het MMP-TIMP systeem. In de afgelopen jaren zijn uitgebreide associaties tussen AGE’s en vasculaire complicaties in diabetes mellitus type 1 beschreven. Daarbij hebben andere studies aangetoond dat blootstelling van cellen aan AGE’s leidde tot een toename van de productie van diverse MMP’s en TIMP-1. Zodoende zouden AGE’s een bijdrage kunnen leveren aan veranderingen in de productie van MMP’s en/of TIMP-1.

Wij hebben specifiek gekeken naar de eiwitgebonden AGE’s; N⁴-(carboxymethyl)lysine (CML), N⁵-(carboxyethyl)lysine (CEL), 5-hydro-5-methylimidazolone (MG-H1) en pentosidine. In onze cross-sectionele analyses hebben we laten zien dat circulerend CML, een ligand voor de receptoren voor AGE’s, positief was geassocieerd met MMP-2 en invers met MMP-9. CEL, een intracellulaire AGE, was positief geassocieerd met MMP-3 en TIMP-1. MG-H1, een ander intracellulaire AGE, was ook geassocieerd met TIMP-1. Pentosidine, een crosslinking AGE, was niet geassocieerd met een van onze markers van het MMP-TIMP systeem. Deze resultaten ondersteunen grotendeels de conclusies uit eerdere, met name in-vitro, onderzoeken en geeft aanwijzingen dat AGE’s mogelijk via MMP’s zouden kunnen leiden tot vasculaire complicaties bij patiënten met diabetes mellitus type 1.

Conclusie

In dit proefschrift hebben we laten zien dat diverse circulerende markers van het MMP-TIMP systeem (MMP-1, -2, -3, en -10 en TIMP-1) geassocieerd zijn, cross-sectioneel danwel prospectief, met diverse vasculaire complicaties en diverse maten van arteriële stijfheid in patiënten met diabetes mellitus type 1. Dit toont aan dat ECM remodelering deel van het pathofysiologisch mechanisme van vasculaire complicaties uitmaakt en dit is nog niet eerder op deze manier aangetoond. Daarnaast hebben we associaties beschreven tussen AGE’s en MMP’s/TIMP-1. De MMP’s en TIMP-1 kunnen dus mogelijk doelen zijn voor preventie en/of behandeling van vasculaire complicaties bij patiënten met diabetes mellitus type 1.