Advanced glycation and type 1 diabetes

Chapter 8

Summary and General discussion

Summary

The increasing prevalence of type 1 diabetes and its related vascular complications pose a tremendous burden for patients and our society (1), but the cellular and molecular mechanisms underlying the development of these vascular complications are still not fully elucidated. For the prevention and/or treatment of these diabetes-related complications, we need a better understanding of these underlying mechanisms. Several potential mechanisms have been proposed to unravel the link between diabetes and the increased risk of cardiovascular disease (CVD) in these patients. We focused on the potential roles of advanced glycation endproducts (AGEs) and their receptor (RAGE), high-mobility group box-1 (HMGB1), endothelial and renal dysfunction, low-grade inflammation (LGI), and arterial stiffness (i.e. pulse pressure, PP).

Present findings

In the EURODIAB PCS nested case-control study we showed that higher levels of sRAGE, as a reflection of higher levels of RAGE, are cross-sectionally associated with greater prevalence of CVD in patients with type 1 diabetes (Chapter 2). This positive association could be attenuated by ~50% by endothelial and renal dysfunction and LGI, which suggests that these mechanisms may constitute pathways that link sRAGE, as a reflection of RAGE, to greater prevalence of CVD in type 1 diabetes. Positive trends were found between higher levels of sRAGE and the presence and across the levels of severity of albuminuria and retinopathy, though these were not independent of traditional risk factors. To gain more insight into the temporal relationship between sRAGE and vascular complications in type 1 diabetes, we investigated the associations between plasma sRAGE and incident fatal and non-fatal CVD as well as all-cause mortality in a 12 year follow-up study (Chapter 3). Incident fatal and non-fatal CVD as well as all-cause mortality doubled per unit higher Ln-sRAGE at baseline. Renal dysfunction, estimated by glomerular filtration rate, could explain in part (up to ~25%) the positive association between plasma sRAGE and incident fatal and non-fatal CVD. In addition, we showed that higher levels of sRAGE at baseline were associated with steeper decline in glomerular filtration rate in the course of follow-up (Chapter 3), which supports the hypothesis that renal dysfunction is an intermediate in the association between sRAGE and cardiovascular disease in these patients.

We measured three AGEs (i.e. CEL, CML, and pentosidine) that could exert unfavourable effects on cellular functions through (partially) different pathways, and together, these may thus reflect a wide range of the potential adverse effects of AGEs. In a 12 year follow-up study in patients with type 1 diabetes, we observed ~30% increase in incident fatal and non-fatal CVD as well as all-cause mortality per 1 unit higher AGEs score (calculated by averaging the z-scores of CEL, CML, and pentosidine) independently of other risk factors (Chapter 4).

To further explore the ligand-RAGE axis, we also investigated the associations between HMGB1, a pro-inflammatory cytokine but also a ligand of RAGE, and the prevalence and incidence of vascular complications in type 1 diabetes. We showed positive cross-sectional associations between serum levels of HMGB1 and the presence and severity of albuminuria, but not with prevalent retinopathy and CVD (Chapter 5). This suggests that serum HMGB1 is involved differently in pathways that lead to micro- and macrovascular complications, which could, at least in part, be explained by the fact that HMGB1 is a ligand for different receptors with distinct functions. However, we cannot neglect the possibility that the lack of association between serum HMGB1 and prevalent CVD was due to the play of chance. In addition, in a prospective study in patients with type 1 diabetes we observed that higher levels of plasma HMGB1 are associated with higher incidence of all-cause mortality (Chapter 6), and, to a lesser extent, also with higher incident fatal and non-fatal CVD. These associations were independent of conventional cardiovascular risk factors and of several potential HMGB1 related pathophysiological mechanisms.

In an attempt to disentangle the associations between LGI (i.e. C-reactive protein, CRP), cross-linking AGEs (i.e. pentosidine), and arterial stiffness as well as their associations with incident fatal and non-fatal CVD (Chapter 7), we showed that in type 1 diabetes, arterial stiffness, as expressed by PP, partially explains the higher risk for CVD associated with higher levels of pentosidine (marker of cross-linking AGEs). Cross-link breakers may, thus, offer a means to reduce arterial stiffening and related CVD in these patients. In addition, PP and CRP were interrelated and explained a portion of the associations with incident CVD in a mutual fashion. Notably, both CRP and PP remained independently associated with higher incidence of CVD, and the combination of elevated CRP with elevated PP increased CVD risk beyond what could be expected from their independent effects, suggesting that LGI and arterial stiffness are causally interrelated and self-enforcing features involved in the development of CVD in type 1 diabetes. Both LGI and arterial stiffness may thus need to be monitored and targeted separately in order to interrupt this vicious cycle and thereby deter the related cardiovascular sequelae often affecting these patients.

General discussion

Study population

We used a European multi-centre nested case-control study and a Danish prospective cohort study in individuals with type 1 diabetes. In the cross-sectional nested case-control study cases were selected as those with the greatest vascular complication burden as possible and controls were selected as those who were completely free of any vascular complication. This selection allowed us to compare individuals with single or multiple complications with individuals free of complications as efficiently as possible. Cases and controls were unmatched, so that the impact of variables of interest could still be assessed and analyses were performed with adjustments for potential confounders.

The Danish prospective cohort study with a 12 year follow-up gave us the opportunity to look at the temporal relationships between determinants of interest and clinical outcomes. This study consisted of 391 patients with type 1 diabetes who were enrolled in a prospective observational study on cardiovascular complications and mortality. In 1993, 199 patients with type 1 diabetes and nephropathy and 192 with normoalbuminuria (i.e. urinary excretion rate <30 mg/24 h) were recruited from the outpatient clinic at Steno Diabetes Center (Gentofte, Denmark). Diabetic nephropathy was defined according to the following clinical criteria: persistent macroalbuminuria >300 mg/24 h in at least two out of three previous consecutive 24-h urine collections, presence of diabetic retinopathy (assessed by fundus photography after pupillary dilatation), and no clinical or laboratory evidence of kidney or renal tract disease other than diabetic glomerulosclerosis.

Both studies included patients with type 1 diabetes only. Therefore, extrapolations to other populations (e.g. patients with type 2 diabetes) need some caution. In addition, because we did not include a control group in our studies, we cannot address the question as to whether the AGE-RAGE axis-related higher prevalence and incidence of vascular complications could explain the excess risk for cardiovascular complications observed in type 1 diabetes.

In aetiological research we try to investigate (confirm or exclude) the relation between a determinant and a certain disease outcome, in which the effects of factors that may obscure such relationship are taken into account. Although the individuals included in our studies were well characterised and we minimised the influence of confounding in the associations observed by adjustments for many possible confounders, interference by factors we did not measure cannot be ruled out. In addition, in all our studies we included individuals in whom full data on variables of interest were available. Selection bias may occur if individuals included in the analyses would differ considerably from the ones excluded from the analyses. However, no such differences with regard to our main determinants or our main outcome

measures were observed between individuals excluded and those included in our studies.

Main determinants

Soluble receptor for advanced glycation endproducts (RAGE)

Different cell types including human endothelial cells express RAGE. In addition to cell-bound RAGE, soluble forms of RAGE (sRAGE) appear extracellularly. These soluble forms result from alternative splicing (2) or from proteolytical cleavage of the membrane-bound RAGE (Figure 1.3), which is most probably shed into the circulation by the sheddase, a disintegrin and metalloprotease 10 (3). The functional role of these soluble forms of RAGE in the circulation remains unclear, but they may reflect the activity of the ligand-RAGE axis. The positive associations observed between sRAGE and prevalence and incidence of CVD reported in Chapter 2 and 3, respectively, are in line with a large Finnish cohort study (4), but not all previous studies (5-7), among patients with type 1 diabetes. The reasons for these contradictory findings are not clear, but may be explained by the fact that different pools of soluble RAGE were measured and investigated across studies, namely, the total pool of sRAGE by the Quantikine sRAGE ELISA kit (R&D systems, Minneapolis, USA) in the Finnish (4) and in our studies, or specifically the splice variant esRAGE by the B-Bridge International esRAGE ELISA kit (Daiichi Fine Chemicals, Takaoka, Japan) in the others (5-7). The inconclusive findings from the studies so far may thus be due to differences in measured variants of soluble RAGE, each of which might have a different function. The differences in the clinical settings or ethnicity might also contribute to the discrepancies in these studies.

The naturally occurring form of sRAGE, as well as artificially produced sRAGE, can potentially bind to a ligand thereby acting as a decoy, which prevents ligand-RAGE interaction and activation (8,9). This could thus explain any inverse association observed between sRAGE/esRAGE and vascular complications. However, although sRAGE has been an interesting subject of investigation under this suspected decoy function, it is very unlikely that sRAGE can act as such (i.e. by capturing and eliminating AGEs and/or other ligands) because the levels of circulating sRAGE are at least 1000 times lower than needed for efficient binding and elimination of AGEs and other ligands. In addition, a recent 4-year prospective multi-centre study in patients with type 2 diabetes has reported both sRAGE and esRAGE to be positively associated with higher risk of coronary heart disease (10).

Advanced glycation endproducts (AGEs)

We included three different AGEs in our analyses. Based on their characteristics each of them may represent a different part of the mechanisms through which AGEs could exert their potential adverse effects in the development of vascular complications in

type 1 diabetes. CEL (as a putative marker of intracellular glycation), CML (as a potential ligand of RAGE), and pentosidine (as one of the cross-linking AGEs), together, may thus reflect a wide range of the potential unfavourable effects of AGEs.

In our study (Chapter 4) we did not normalise CEL, CML and pentosidine for an amino acid, e.g. lysine, and this may not enable direct comparison (of absolute values) with other studies that have done so (and did not report the values for the AGEs and the amino acid, separately). This would be an important limitation if such lack of normalisation would affect our findings. In order to address this, we have examined data from the EURODIAB study, in which plasma CML, CEL, and lysine concentrations were available (11). When we examine the correlations between CEL and CML with or without normalisation for lysine (39.7 ± 4.4 mM), we found that CEL (1.21 ± 0.47 μM) and CEL/lysine (31 ± 11 μM/M) as well as CML (2.26 ± 0.79 μM) and CML/Lysine (57 ± 18 μM/M) were highly correlated: correlation coefficients were 0.95 and 0.94, respectively. Moreover, in the EURODIAB study the associations between plasma CEL as well as CML and the presence of CVD did not differ between normalised and non-normalised values. Given these strong correlations between normalised and non-normalised values of AGEs, and their comparable associations with the presence of CVD, normalisation for an amino acid concentration is unlikely to affect the associations between plasma AGEs and study endpoints in our prospective study (Chapter 4).

High-mobility group box-1 (HMGB1)

HMGB1 was discovered as an intranuclear protein involved in the DNA organisation and regulation of transcription (12). Recently, extracellular HMGB1, released by necrotic cells (13) and inflammatory cells upon activation (14), has been suggested to function as a pro-inflammatory cytokine (15), at least in part, through ligation of RAGE (16) or toll-like receptors (17).

We have measured HMGB1 levels in serum in the cross-sectional study and in plasma in the prospective study. In the cross-sectional study we did not find a positive association between serum HMGB1 and prevalent CVD (Chapter 5) in contrary to the positive association observed in the prospective study between plasma HMGB1 levels and incident CVD (Chapter 6). The lack of association between serum HMGB1 and prevalent CVD may be due to the play of chance or residual confounding by, for example, lipid lowering drugs. However, the apparent discrepancy could also imply that *serum* HMGB1 and *plasma* HMGB1 do not represent the same pool of HMGB1 in these patients. This might be explained by the platelet activation and coagulation cascade initiated in the vacuum container during the usual process to obtain serum samples from the blood samples. Activated platelets release several pro-inflammatory cytokines, and platelet activation markers are positively associated with serum HMGB-1 levels in patients with disseminated intravascular coagulation associated with haematologic malignancies (18). In addition, it has been reported that part of the HMGB-1 present in the cytoplasma of resting platelets could be transported to the

platelet surface upon platelet activation (19). This process may vary per blood sample according to the platelet count and, therefore, serum HMGB-1 levels may not reflect adequately the extracellular HMGB-1 levels in the subjects.

Circulating levels vs. tissue levels of biomarkers

In our studies we have measured circulating levels of sRAGE, AGEs, and HMGB1 only, and it is not clear whether these are representative of the total pool of these biomarkers. Indeed, AGEs accumulate in tissue and cellular concentrations of AGEs are higher than plasma AGEs levels (23). The extents to which the circulating levels of RAGE and HMGB1 relate to their tissue levels are unknown. Further studies in which both circulating and tissue levels of these biomarkers are measured are needed to clarify the relation between the biomarkers levels in the two compartments and their specific associations to vascular complications.

Low-grade inflammation and endothelial dysfunction

We used biomarkers to represent LGI and endothelial dysfunction, though we do not exactly know whether this is the most adequate method to represent these pathophysiological mechanisms, because both mechanisms comprise many aspects.

In addition, the levels of the markers of LGI and endothelial dysfunction investigated were only measured once, which might have diluted the associations we found. However, we combined the z-scores of the different markers of LGI and endothelial dysfunction into averaged scores to overcome biological variability of a single biomarker. A z-score represents the distance between an individual's biomarker score from the population's total mean in units of the population's standard deviation, and is thus a common transformation that enables the combination of several markers originally expressed in different units. We assumed that every biomarker measured reflects (part of) the process of LGI or endothelial dysfunction. We have weighted them equally, however, it is unclear which marker may be more important than the others.

Arterial stiffness (i.e. pulse pressure)

Brachial PP was calculated on the basis of two blood pressure readings, which could be affected by measurement error. In addition, we used brachial, not central, PP as an estimate of arterial stiffness. In a recent meta-analysis, both central and brachial PP (10 mmHg) were significantly associated with incident CVD and mortality [HR=1.32 (1.22-1.42) and HR=1.19 (1.10-1.28), respectively] (20). Although central PP had only marginal added value in CVD risk prediction, the magnitude of the risk estimates was higher for the central than the brachial PP. A recent study in type 1 diabetes (the FinnDiane Study) has shown that both brachial (10 mmHg) [risk-factors adjusted HR=1.22 (1.10-1.34) as estimated in the whole study population: events/n=178/4,321] and central PP [risk-factors adjusted HR=1.29 (1.02-1.62), but estimated in a sub-

population only: events/n=35/408], were associated with incident CVD, though the latter association was no longer significant after adjustment for previous CVD (21). Nevertheless, and despite the need to move beyond brachial PP to better understand aetiology and fine tune stiffness-related risk estimation, it remains that brachial PP is an easily accessible measure and current clinical practice and decision making still relies on brachial blood pressure recordings. Furthermore, given the evidence for accelerated arterial aging in type 1 diabetes (22), the central-to-brachial PP amplification phenomenon may be less influential in these individuals.

Outcomes

In the EURODIAB study prevalent CVD was defined as a positive medical history of a cardiovascular event including myocardial infarction, angina, coronary artery bypass graft and/or stroke, and/or ischaemic changes on a centrally Minnesota-coded ECG. This non-fatal outcome may be susceptible to misclassifications, because of its dependency on the completeness and accuracy of patients' records and/or discharge letters. Albumin excretion rates (UAEs) were measured from duplicate 24 h urine collections, which are considered the gold standard for measuring albuminuria. However, these collections are cumbersome and inadequate collections may impair its reliability. Retinopathy was assessed from retinal photo-graphs according to the EURODIAB protocol (2-field 45° retinal photography) (24). This method compared favourably with the gold standard 7-field 30° retinal photography. The 2-field 45° proved to be acceptably repeatable and accurate (24).

In the Danish prospective cohort study all patients were traced through the national register, which is a reliable source for mortality dates. The date of death was recorded and information on the primary cause of death was obtained from the death certificate, which was reviewed by two independent observers. Additional available information from necropsy reports was also included. In all patients alive at the end of follow-up non-fatal cardiovascular events were retrieved from their patient files from Steno Diabetes Center and/or other hospital records. The primary study outcome was a combined end-point of fatal and non-fatal CVD (i.e. myocardial infarction, percutaneous coronary intervention, coronary bypass grafting, amputation due to ischaemia, vascular surgery for peripheral atherosclerotic disease and stroke), and the secondary outcome was all-cause mortality. All deaths were classified as cardiovascular unless an unequivocal non-cardiovascular cause was established. Potential misclassification of non-specific mortality as CVD-related mortality may have introduced non-differential biases, in which case the estimates reported herein may have been underestimated.

Although the secondary end-point in this study (all-cause mortality) was quite valid, our primary end-point including non-fatal end-points may have been more susceptible to misclassification bias, because of its dependency on the completeness and accuracy of patients' records and/or discharge letters. Most likely, we may have

missed some cases (i.e. patients who were possibly considered as 'free from CVD' when in fact they were not), but then such misclassification was likely to be random because CVD status was ascribed without prior knowledge of subjects' biomarkers levels. Again, these misclassifications, if present, may, if anything, have led to an underestimation of the strength of the associations between our main determinants and the combined main study end-point (fatal and non-fatal CVD).

Clinical relevance

The increasing prevalence of type 1 diabetes and its related vascular complications pose a tremendous burden for patients and our society, while the pathophysiological mechanisms underlying the development of these vascular complications are still to be elucidated. Results from animal studies suggested that the AGE-RAGE axis (9,23,25,26) and HMGB-1 (27) may play a role in the development of vascular complications in diabetes. Whether these animal models could adequately reflect the human clinical situation is unclear. Beside the differences between species, we also cannot neglect the differences in settings (28). Namely, in experimental animal studies the animal model is carefully selected to investigate one specific determinant, while in a human clinical setting we are confronted with patients with several comorbidities and various circumstances. Translational observational/descriptive studies in humans are often limited by ethical and financial issues. Ideally, we would like to test our hypotheses at relevant sites where the pathophysiological processes take place. However, mostly we are restricted to investigate markers, such as circulating levels, of the determinants of interest. Despite of this limitation, the findings observed in our studies have additional value to the existing knowledge from animal studies and bring us a step closer to revealing the relevance of the pathophysiological mechanisms investigated to the higher risk for vascular complications in type 1 diabetes. In addition, we have tried not to look only for putative associations between determinants and outcomes, but also to provide insights into the underlying pathways. Furthermore, though the intention of our studies was to investigate aetiological hypotheses, circulating levels of biomarkers investigated may have predictive qualities and could form easy accessible clinical tools, in contrast to the more difficultly obtainable tissue levels of these biomarkers.

We showed that sRAGE, AGEs, HMGB1, and markers of LGI and arterial stiffness were, independently of traditional risk factors, associated with greater prevalence and/or higher incidence of vascular complications in patients with type 1 diabetes. These risk factors could constitute new targets in the prevention and/or treatment of vascular complications in type 1 diabetes. In addition, we showed that the individual mechanisms are related to each other, as illustrated in Figure 8.1. Higher incident CVD associated with higher levels of plasma sRAGE may, in part, be explained by sRAGE-associated renal dysfunction. In addition, increased arterial stiffness may constitute a mechanism through which cross-linking AGEs could lead to higher risk for CVD, and

therefore, targeting cross-linking AGEs may offer a means to reduce related arterial stiffening and CVD in type 1 diabetes. Furthermore, both CRP and PP were independently associated with higher incidence of CVD, and the combination of elevated CRP with elevated PP increased CVD risk beyond what could be expected by their independent effects, suggesting that LGI and arterial stiffness exert self-enforcing effects on CVD in type 1 diabetes. This suggests that both LGI and arterial stiffness may need to be monitored and targeted separately in order to interrupt this vicious cycle and thereby deter the related cardiovascular sequelae often affecting these patients.

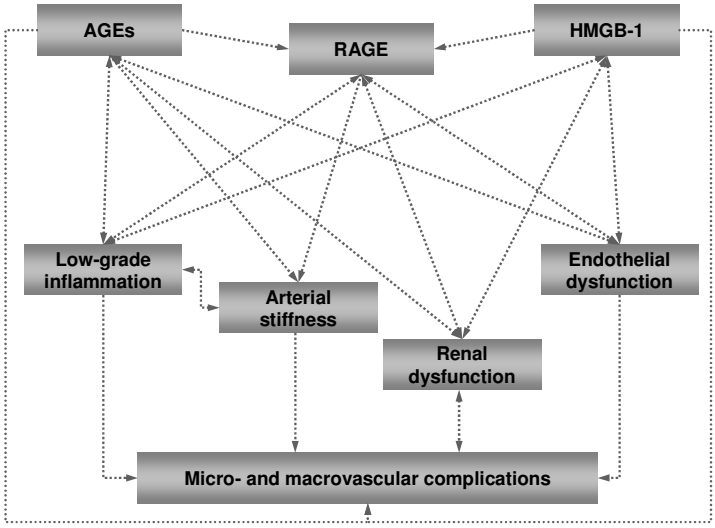

Figure 8.1. Present findings.

Future research

Although markers of the AGE-RAGE axis, LGI and arterial stiffness were associated with greater prevalence and/or higher incidence of vascular complications in patients with type 1 diabetes, longitudinal studies are needed to elucidate further the temporal relationship between these risk factors and clinical outcome. In addition, for our mediation analyses we had data at one time point only on the determinants and potential mediators and, thus, we cannot exclude the possibility of reverse causation as illustrated by the bi-directional arrows in Figure 8.1 between our main determinants (i.e. AGEs, sRAGE, and HMGB1) and our potential mediators (i.e. LGI, endothelial and renal dysfunction, and arterial stiffness). For more sophisticated

mediation analyses, in which potential reverse causation analyses could be better addressed we would need data on both determinants and potential mediators at different time points during follow-up. No longitudinal data of the associations observed exist in large groups of type 1 diabetes at the time, thus these studies may serve as a reasonable starting point to further explore these associations.

In addition, studies in which both circulating and tissue levels of these biomarkers are measured are needed to clarify the relation between the biomarkers levels in the two compartments and their specific relevance to vascular complications.

Furthermore, it remains to be proven whether targeting and lowering the levels of these risk factors could also result in a more favourable clinical outcome. To enable such intervention studies we would also need drugs that are able to decrease levels and/or activity of the AGE-RAGE axis, LGI, and arterial stiffness.

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Samenvatting

Samenvatting

Type 1-diabetes is een chronische ziekte, die zich gewoonlijk presenteert in de kindertijd of in de adolescentie. Type 1-diabetes wordt gekenmerkt door hoge glucosewaardes (hyperglykemie) en ontstaat door (auto-immuun) vernietiging van de insuline-producerende beta-cellen geclusterd in de eilandjes van Langerhans van de alvleesklier, wat resulteert in veelal een absoluut tekort aan insuline die dagelijkse insuline toediening noodzaakt. Symptomen veroorzaakt door hyperglykemie zijn onder meer polyurie, polydipsie, gewichtsverlies, verandering van gezichtsvermogen en vermoeidheid. Het aantal nieuwe ziektegevallen (incidentie) van type 1-diabetes bij kinderen varieert wereldwijd van 0,1/100.000 per jaar in Zunyi, China tot 36,8/100.000 per jaar in Sardinië, Italië. In Nederland wordt de incidentie van type 1-diabetes geschat op 13/100.000 per jaar bij kinderen ≤14 jaar. De incidentie van type 1-diabetes lijkt toe te nemen in alle leeftijdsgroepen, met name bij jongere kinderen, met een totale jaarlijkse stijging van ~3,9% geschat op basis van 20 populatie-gebaseerde registers in 17 Europese landen in de periode 1989-2003. De redenen voor deze toenemende incidentie zijn onbekend, maar waarschijnlijk spelen omgevingsfactoren een rol hierin. Ondanks uitgebreid wetenschappelijk onderzoek, kan type 1-diabetes niet worden voorkomen of genezen met de huidige beschikbare behandelingen, zoals immunotherapie, stamceltherapie of transplantatie van eilandjes van Langerhans.

Wereldwijd zijn er ~22 miljoen patiënten met type 1-diabetes en dit zal naar verwachting verdubbelen in de komende 20-30 jaar. In Nederland wordt het huidige voorkomen (prevalentie) van type 1-diabetes geschat op 100.000. De last van diabetes voor zowel patiënten als voor onze maatschappij wordt voornamelijk veroorzaakt door diabetes-gerelateerde korte en lange termijn complicaties, geïllustreerd door een ~4-voudige toename in het risico op hart- en vaatziekten (HVZ) bij type 1-diabetes, en een ~7-voudige en ~17-voudige toename van het risico op sterfte aan HVZ bij respectievelijk mannen en vrouwen, die type 1-diabetes hadden en jonger dan 40 jaar waren, vergeleken met mensen zonder diabetes. Daarnaast leiden diabetes-gerelateerde microvasculaire complicaties (bijvoorbeeld diabetische retinopathie en nefropathie) tot een vermindering van visus en nierfunctie, die een hogere morbiditeit met zich meebrengen bij patiënten met type 1-diabetes.

De toenemende prevalentie van type 1-diabetes en de bijbehorende vasculaire complicaties vormen een enorme last voor de patiënten en voor onze samenleving, maar de cellulaire en moleculaire mechanismen die ten grondslag liggen aan het ontstaan van deze vasculaire complicaties zijn nog steeds niet volledig opgehelderd. Voor de preventie en/of de behandeling van deze diabetes-gerelateerde complicaties, moeten we een beter begrip verkrijgen van de mogelijk onderliggende mechanismen. Verschillende mechanismen zijn geopperd om de link tussen diabetes en het verhoogde risico op HVZ bij deze patiënten te verklaren. We hebben ons gericht op de potentiële rol van geavanceerde glycerings eindproducten (AGEs) en hun receptor

(RAGE), high-mobility group box-1 (HMGB1), endotheel- en nierfunctiestoornissen, laaggradige ontsteking, en arteriële stijfheid (gemeten als verhoogde polsdruk).

Resultaten

In de EURODIAB nested case-control studie hebben we geobserveerd dat hogere waardes van het in plasma circulerende RAGE (sRAGE), als een weerspiegeling van hogere waardes van RAGE, cross-sectioneel geassocieerd zijn met een hogere prevalentie van HVZ in patiënten met type 1-diabetes (hoofdstuk 2). De sterkte van deze associatie neemt met ~50% af wanneer wij de rol van endotheel- en nierdisfunctie en laaggradige ontsteking in het model opnemen, hetgeen suggereert dat deze mechanismen mogelijk de link kunnen vormen tussen hogere waardes van sRAGE, als een reflectie van RAGE, en de hogere prevalentie van HVZ in patiënten met type 1-diabetes. Positieve trends werden ook gevonden tussen hogere waardes van sRAGE en de aanwezigheid als ook de mate van ernst van albuminurie en retinopathie, maar deze waren niet onafhankelijk van de traditionele risicofactoren van HVZ. Om meer inzicht te krijgen in de temporele relatie tussen sRAGE en vasculaire complicaties bij type 1-diabetes, onderzochten we de associatie tussen plasma sRAGE waardes en de incidentie van fatale en niet-fatale gevallen van HVZ evenals de totale sterftegevallen in een studie waarin we de patiënten 12 jaar hebben gevolgd (hoofdstuk 3). Incidentie van fatale en niet-fatale HVZ alsook totale mortaliteit verdubbelde ongeveer per eenheid hogere waarde van plasma sRAGE, welke is gemeten aan het begin van de studie. Nierdisfunctie, geschat door glomerulaire filtratiesnelheid, zou hierbij voor een deel (tot ~25%) de positieve associatie tussen plasma sRAGE en incidentie van fatale en niet-fatale HVZ kunnen verklaren. Daarnaast toonden we aan dat hogere waardes van plasma sRAGE gemeten bij aanvang van de studie geassocieerd waren met sterkere daling van de glomerulaire filtratiesnelheid gemeten in de loop van de studie (hoofdstuk 3), welke de hypothese ondersteunt dat nierfunctiestoornis een intermediaire rol kan spelen in de associatie tussen sRAGE en HVZ in deze patiënten.

We hebben drie AGEs (CEL, CML, en pentosidine) gemeten, die ongunstige effecten op de celfuncties zouden kunnen uitoefenen door (gedeeltelijk) verschillende mechanismen, en samen, zouden deze drie dus een breed scala van de mogelijke schadelijke effecten van AGEs kunnen weerspiegelen. In een 12 jaar follow-up studie in patiënten met type 1-diabetes, observeerden we een ~30% toename in incidentie van fatale en niet-fatale HVZ alsook totale mortaliteit per 1 eenheid hogere AGEs score (berekend als het gemiddelde van de z-scores van CEL, CML, en pentosidine) en deze associatie was onafhankelijk van andere HVZ risicofactoren (hoofdstuk 4).

Om verder de rol van de RAGE-as te verhelderen, hebben we ook onderzoek gedaan naar HMGB1, een pro-inflammatoir cytokine die een rol speelt bij ontsteking als deel van ons afweersysteem, maar ook een substraat die zich aan RAGE kan binden en kan activeren. We onderzochten de associaties tussen HMGB1 en de prevalentie

en incidentie van vasculaire complicaties bij type 1-diabetes. We observeerden een cross-sectionele associatie tussen hogere serumspiegels van HMGB1 en de aanwezigheid en de ernst van albuminurie, maar niet met retinopathie of HVZ (hoofdstuk 5). Dit suggereert dat serum HMGB1 op andere manieren betrokken kan zijn in de mechanismen die leiden tot micro- en macrovasculaire complicaties, welke ten dele verklaard zouden kunnen worden door het feit dat HMGB1 kan binden aan verschillende receptoren met verschillende functies. We kunnen echter niet voorbijgaan aan de mogelijkheid dat het ontbreken van een associatie tussen serum HMGB1 en prevalentie van HVZ te wijten was aan toeval. Aangezien wij in de prospectieve studie in patiënten met type 1-diabetes wel een associatie observeerden, waarbij hogere waardes van plasma HMGB1 geassocieerd waren met een hogere incidentie van totale mortaliteit (hoofdstuk 6) en, in een minder sterke mate, ook met hogere incidentie van fatale en niet-fatale HVZ. Deze associaties waren onafhankelijk van conventionele HVZ risicofactoren en verschillende potentiële HMGB1 gerelateerde pathofysiologische mechanismen.

In een poging om de associaties tussen laaggradige ontsteking (gerepresenteerd door C-reactive protein, CRP), cross-linking AGEs (gerepresenteerd door pentosidine), en arteriële stijfheid onderling alsmede hun associaties met de incidentie van fatale en niet-fatale HVZ op te helderen (hoofdstuk 7), observeerden we in patiënten met type 1-diabetes, dat arteriële stijfheid, gerepresenteerd door polsdruk, gedeeltelijk het hogere risico op HVZ gerelateerd aan hogere pentosidinewaardes (marker van cross-linking AGEs) kon verklaren. Cross-link brekers kunnen daardoor een middel vormen om arteriële verstijving en de daaraan gerelateerde HVZ in deze patiënten verminderen. Daarnaast gingen hogere polsdrukwaardes samen met hogere CRP waardes, waarbij ze wederzijds hun associatie met een hogere incidentie van HVZ deels konden verklaren. Belangrijk daarbij is dat zowel hogere waardes van polsdruk als CRP onafhankelijk van elkaar geassocieerd bleven met hogere incidentie van HVZ en de combinatie van hoge polsdruk met hoog CRP het risico op HVZ vergrootte boven het te verwachten risico van wanneer men hun afzonderlijk effecten bijelkaar zou optellen. Dit suggereert dat arteriële stijfheid en laaggradige ontsteking causaal samenhangen en tevens dat er elkaar onderling versterkende mechanismen betrokken zijn bij het ontstaan van HVZ in type 1-diabetes. Zowel arteriële stijfheid als laaggradige ontsteking dienen dus afzonderlijk gemonitord en behandeld te worden om deze vicieuze cirkel te onderbreken en daarmee de cardiovasculaire complicaties die vaak in deze patiënten optreden te verminderen.

In hoofdstuk 8 worden de belangrijkste bevindingen van dit proefschrift samengevat en bediscussieerd. Ondanks meerdere beperkingen, zijn de bevindingen in onze studies van additionele waarde boven de bestaande kennis vanuit voornamelijk dierproeven en brengen deze observaties ons een stap dichter bij het ophelderen van de relevantie van de onderzochte pathofysiologische mechanismen ter verklaring van het hogere risico op vasculaire complicaties geassocieerd met type 1-diabetes. Daarnaast hebben we geprobeerd niet uitsluitend de mogelijke associaties

tussen determinanten en uitkomsten te benoemen, maar ook om inzicht te verschaffen in de potentiële onderliggende mechanismen als link tussen determinant en uitkomst.