Cardiovascular morbidity and mortality in diabetes mellitus: Prediction and prognosis

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THE EIGHT ORIGINAL PAPERS ARE

1. Astrup AS, Tarnow L, Rossing P, Pietraszek L, Hansen PR, Parving H-H. Improved prognosis in type 1 diabetic patients with nephropathy: A prospective follow-up study. Kidney Int 2005; 68(3):1250-1257.
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3. Astrup AS, Tarnow L, Rossing P, Hansen BV, Hilsted J, Parving H-H. Cardiac autonomic neuropathy predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. Diabetes Care 2006; 29(2):334-339.
4. Astrup AS, Tarnow L, Christiansen M, Hansen PR, Parving H-H, Rossing P. Pregnancy-associated plasma protein A in a large cohort of Type 1 diabetic patients with and without diabetic nephropathy-a prospective follow--up study. Diabet Med 2007; 24(12):1381-1385.
5. Astrup AS, Tarnow L, Pietraszek L et al. Markers of endothelial dysfunction and inflammation in type 1 diabetic patients with or without diabetic nephropathy followed for 10 years: association with mortality and decline of glomerular filtration rate. Diabetes Care 2008; 31(6):1170-1176.
6. Kim WY, Astrup AS, Stuber M et al. Subclinical coronary and aortic atherosclerosis detected by magnetic resonance imaging in type 1 diabetes with and without diabetic nephropathy. Circulation 2007; 115(2):228-235.
7. Astrup AS, Kim WY, Tarnow L et al. Relation of left ventricular function, mass, and volume to NT-proBNP in type 1 diabetic patients. Diabetes Care 2008; 31(5):968-970.
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INTRODUCTION

The prevalence of diabetes mellitus is increasing worldwide, and although this is primarily due to an increase in the incidence of type 2 diabetes, the incidence of type 1 diabetes is also increasing (9;10). Diabetic nephropathy is a leading cause of diabetes related mortality (11), however the causes of mortality and morbidity in patients with diabetic nephropathy is changing. Today patients with diabetic nephropathy live longer due to medications postponing ESRD, and due to the possibility of treating ESRD with a renal transplant or dialysis. This have changed the causes of death in patients with nephropathy, and today the major concern is not just preventing ESRD but also preventing cardiovascular disease. Identifying patients at high risk, and finding predictors of disease, is beneficial in order to start early intervention. Furthermore, identifying patophysiological mechanisms are necessary to find new treatment modalities. The studies in this thesis has focused on describing the prognosis in patients with diabetic nephropathy today and the evaluation of various cardiovascular risk factors and markers for all-cause mortality and cardiovascular morbidity and mortality. The studies evaluated patophysiological mechanisms, and looked at mechanisms involving endothelial dysfunction and low-grade inflammation, and the link to developing cardiovascular disease, and their involvement in progression of renal disease. Finally, by using cardiac magnetic resonance, we evaluated the prevalence of cardiovascular disease in type 1 diabetic patients, and non-invasively visualized the structure and function in the heart and its vessels in patients with an increased cardiovascular risk.

The major aims of this thesis were:

To evaluate cardiovascular risk factors and to asses the current prognosis in patients with type 1 diabetes and diabetic nephropathy:
The current prognosis in type 1 diabetic patients with diabetic nephropathy was evaluated in a prospective observational study with 10 years follow-up, including 401 type diabetic patients with or without diabetic nephropathy (12). The cumulative incidence of

cardiovascular disease during follow-up was assessed, and various risk factors and markers including PAPP-A, PlGF, as well as a set of markers for low-grade inflammation and endothelial dysfunction, and cardiac autonomic neuropathy were evaluated. Finally, the relation between rate of decline of GFR to biomarkers and to autonomic neuropathy was examined.

To investigate the prevalence of cardiovascular disease in patients with type 1 diabetes with or without diabetic nephropathy:

136 patients with type 1 diabetes hereof 63 patients with diabetic nephropathy and 73 patients with persistent normoalbuminuria, all without symptoms of CVD, were included in a case control study. All patients had performed a clinical investigation, and a cardiac magnetic resonance scan. The study evaluated silent heart disease, coronary stenosis, plaque burden, left ventricular mass, function, and volumes, and relation to NT-proBNP.

To investigate cardiovascular risk factors in patients with type 2 diabetes, and to evaluate the prognostic value of the non-dipping phenomenon:

In a long-term follow-up study a cohort of 104 patients with type 2 diabetes (13) hereof 51 patients with diabetic nephropathy were followed for 13 years. The population provided an opportunity to study risk factors carefully evaluated at baseline including LVH, HRV, and 24 h ambulatory blood pressure. The 24 h ambulatory blood pressure made it possible to evaluate the prognostic importance of the non-dipping phenomenon, which to our knowledge not previously has been studied before in a long-term follow-up study in patients with diabetes. The non-dipping phenomenon could not be evaluated in our cohorts of type 1 diabetic patients.

STUDY POPULATIONS

For evaluation of cardiovascular risk factors and to asses the current prognosis in patients with type 1 diabetes and diabetic nephropathy:

For the study of traditional and new risk factors for all-cause mortality and cardiovascular mortality and morbidity in type 1 diabetic patients a case-control study including 200 patients with type 1 diabetes and diabetic nephropathy and 201 patients with persistent normoalbuminuria was initiated in 1993. Originally, in 1993 all patients with type 1 diabetes and diabetic nephropathy at the Steno Diabetes Center who had their glomerular filtration rate measured the same year was invited to participate. The 200 patients with diabetic nephropathy who accepted and thus was enrolled as cases was an unbiased sample of the whole group of 242 patients eligible for the study (12). Patients in the control group all had persistent normoalbuminuria, and were matched to cases for age, gender and duration of diabetes. During follow-up, 60 patients with nephropathy died compared to 16 patients with normoalbuminuria.

The incidence of diabetic nephropathy declines to approximately 1 % pr year in long-standing diabetes (14-17) and thus all patients in the control group were selected on the criteria of a long diabetes duration. At the baseline examination in 1993 all patients in the control group had long-standing diabetes with a mean diabetes duration of 26 years (SD 9), ranging from 15-55 years. At the follow-up examination in 2003, 13 patients had developed microalbuminuria; however no patients had progressed to overt diabetic nephropathy. All patients were examined at follow-up with a questionnaire and a clinical investigation, including an exercise-ECG.

For investigation of the prevalence of cardiovascular disease in patients with type 1 diabetes with or without diabetic nephropathy:

To evaluate subclinical coronary artery disease, left ventricular function, mass, and dimensions and relation to NT-proBNP in patients with type 1 diabetes and with or without diabetic nephropathy, we conducted a cross-sectional study. From July 2003 to February 2005, 136 patients from the Steno Diabetes Center with type 1 diabetes were included, hereof 63 (46%) patients with diabetic nephropathy and 73 patients with persistent normoalbuminuria. All patients were without symptoms or clinical history of cardiovascular disease. Both groups were representative of a random selection of patients at the Steno Diabetes Center, recruited from approximately 3000 subjects with type 1 diabetes (herein 400 patients with nephropathy), however patients with known cardiovascular disease were excluded. Patient files were examined and patients were invited to participate if the criteria for inclusion in the study were fulfilled: diabetes duration longer than 15 years in patients with normoalbuminuria, to make sure that this group will have persistent normoalbuminuria, and in all patients no evidence of cardiovascular disease according to a WHO questionnaire (15). The only exclusion criteria were those related to CMR safety or mental illness, or known cardiovascular disease. Furthermore, all patients were examined with a questionnaire and a clinical investigation, including an exercise-ECG.

For investigation of cardiovascular risk factors in patients with type 2 diabetes, and for evaluation of the prognostic value of the non-dipping phenomenon:

To evaluate cardiovascular risk factors and the importance of 24-hour blood pressure variability in type 2 diabetic patients we identified a cohort of Caucasian type 2 diabetic patients in 1991 (13). In 2004 the cohort was followed up in regards to all-cause mortality, and the prognostic importances of baseline parameters were evaluated. A total of 104 patients were included, hereof 51 with diabetic nephropathy. The control group consisted of 53 patients with type 2 diabetes and normoalbuminuria who were matched to cases for gender, age, and known diabetes duration. All patients met the criteria for type 2 diabetes according to the WHO guidelines (18). During the 13 years of follow-up 41 patients with nephropathy died and 13 patients with normoalbuminuria died.

DEFINITIONS AND CLINICAL ENDPOINTS

Diabetic nephropathy and normoalbuminuria:

Persistent albuminuria was defined as UAER above 300 mg/24 h in at least two of three consecutive samples (19). Diabetic nephropathy was defined according to accepted clinical criteria: persistent albuminuria > 300 mg/24 h in two of three consecutive determinations, presence of diabetic retinopathy and no evidence of other kidney or renal tract disease (19;20). Time for onset of diabetic nephropathy was defined as the first recorded positive urine sample in at least two of three consecutive samples. Normoalbuminuria was defined as urinary albumin excretion rate ≤ 30 mg/24 h.

Endpoints:
ESRD was defined as renal transplant, dialysis, or having a creatinine value above 500 µmol/l within the last year before time of death. This definition allowed us to compare our data with historic data before treatment with dialysis and renal transplantation was possible.
For cardiovascular mortality and morbidity a combined endpoint was used consisting of cardiovascular death, history of nonfatal myocardial infarction, percutanous coronary intervention (PCI), coronary artery bypass grafting (CABG), nonfatal stroke, amputation as a result of ischemia, and vascular surgery for peripheral atherosclerotic disease (PAD). The same endpoints were used in the Steno-2 trial (21). Cardiovascular death was classified as all deaths for which an unequivocal non-cardiovascular cause was not established (22).

METHODS

Ambulatory blood pressure:
In all studies, 24-h ambulatory blood pressure measurements were measured with a TADEKA TM 2420/2421 device 6 and 7. The device has previously been validated to satisfaction (23;24). Blood pressure was measured every 15 minutes during the day (7:00 a.m. to 23:00 p.m.) and every 30 minutes throughout the night (23:00 p.m. to 7:00 a.m.). Blood pressures were averaged for each hour before calculating the 24-hour blood pressure. All devices were routinely calibrated by a local A & D agent and devices had a variation of less than ±3 mmHg for both systolic and diastolic blood pressure. Recordings from our clinic of 24-hour ambulatory blood pressure on two occasions 2-4 month apart in 63 diabetic patients gave the following coefficients of variation for systolic/diastolic blood pressure: 24-hour: 10% / 8%, daytime: 10% / 7%, and night-time: 13% / 13%.
There has been a number of different ways to define abnormal blood pressure variation. We used the relative change in systolic and diastolic blood pressure as a continuous variable, and dippers were defined as subjects with an average reduction in systolic and diastolic blood pressure ≥ 10% from day to night (25;26). Dipping of night blood pressure was calculated from the average night and day blood pressures from a 24-hour ambulatory blood pressure measurement:
((systolic blood pressure day – systolic blood pressure night)/systolic blood pressure day)+
(diastolic blood pressure day – diastolic blood pressure night)/diastolic blood pressure day))/2.
It was more recently suggested that a reduction of 0% was close to the 95% upper percentile of normotensive subjects (27), meaning that 5% of patients without hypertension have a higher blood pressure during night-time than during daytime (reversed dipping). We used this definition of reversed dipping in our study.

Kidney function:
To estimate kidney function in all type 1 diabetic patients with diabetic nephropathy and in all patients with type 2 diabetic patients glomerular filtration rate (GFR) was measured in supine position after a single injection of edetic acid labelled with 3.7 MBq sodium 51chromate in the morning by determining the radioactivity in venous blood samples 180, 200, 220, and 240 minutes after injection (28;29). The underestimation (10%) of 51Cr-EDTA clearance vs. inulin clearance (30) was corrected for by multiplying the 51Cr-EDTA clearance by 1.10. Extra-renal loss was corrected for by subtracting 3.7 ml/min. Finally, we standardised for 1.73 m2 body surface area using the patient's body surface area at baseline and used this throughout the study period. Linear regression analysis, least square method, was used to determine the rate of decline in GFR in two papers looking at the relation between rate of decline in GFR and autonomic neuropathy and biomarkers (3;5). We included patients in this analysis if we had at least three GFR measurements during follow-up.

Cardiac autonomic neuropathy:
Diagnostic tests of cardiac autonomic neuropathy (CAN) includes resting heart rate (> 100 beats per minute is abnormal), heart rate variation (HRV) (≤ 10 beats/min is abnormal), heart rate response to standing and to Valsalva maneuver, systolic blood pressure response to standing (abnormal blood pressure fall is above 30 mmHg), diastolic blood pressure response to isometric exercise where the subject squeezes a handgrip and diastolic blood pressure in the other arm is measured (normal rise is above 16 mmHg in the other arm), ECG QT/QTc intervals (> 440 ms is abnormal), spectral analysis, and measurements of neurovascular flow (31). We only used HRV as one simple method to measure CAN and to categorize patients into normal or abnormal function of the cardiac autonomic nervous system. This is of course a limitation and it is recommended to use three different tests addressing R-R interval, Valsalva maneuver, and postural blood pressure testing in order to determine the severity of CAN (32). However in a metaanalysis CAN measured by HRV was strongly associated with increased risk of silent myocardial infarction and mortality (33). Furthermore, our patient population is well characterized and we have followed the cohorts for a long time.
We assessed HRV by Expiration/Inspiration (E/I) variation in heart rate according to the method described by Hilsted et al (34). To perform the test the patient was in supine position asked to breathe deeply at the rate of 6 breaths per minute for one minute while being monitored by electrocardiogram. The maximum and minimum heart rates during each breathing cycle were measured, and the mean of the differences were calculated. The cardiac autonomic function was defined as abnormal when HRV ≤ 10 beats/min at baseline, as originally suggested by Ewing as abnormal (35). The method is simple and of low cost which makes it suitable for evaluating CAN in a large group of patients.

Evaluation of cardiovascular disease by ECG and CMR:
To evaluate cardiovascular disease (CVD) and history of CVD in a standardized way at the clinical examination at Steno Diabetes Center we used a WHO cardiovascular questionnaire together with a resting 12-lead electrocardiogram (ECG). The ECG was subsequently read independently by two trained observers, who were masked to the clinical status of the patients, using the Minnesota Rating Scale (36). CVD was diagnosed if the ECG showed signs of probable myocardial infarction (Minnesota code 1.1-2) or possible myocardial ischemia (Minnesota Rating Scale 1.3, 4.1-4, 5.1-3, and 7. 1).
All patients without history of CVD who were capable of sitting on an exercise bike performed an exercise ECG. The test was carried out in accordance to guidelines from the Danish Society of Cardiology (37). Test results were analyzed by a masked cardiologist (Peter Riis Hansen) and classified into pathological test, normal test, or inconclusive test. The test was defined pathological if the ECG showed exercise-induced ST-depression ≥ 2 mm, ventricular arrhythmia during exercise, or development of left branch bundle block. The test was considered inconclusive if the patient failed to reach 85% of the calculated theoretical maximum heart rate (220-patient age), and the ECG was without pathological findings.

The exercise-ECG was not very suitable for patients with diabetic nephropathy, since a large proportion (47 %) of subjects with nephropathy was unable to achieve 85% of their maximum predicted heart rate with exercise resulting in inconclusive test results.
Therefore we wished to extend the evaluation of CVD in asymptomatic type 1 diabetic patients since the follow-up study showed a 40 percent risk over 10 years for development of CVD in patients with diabetic nephropathy and a 10 percent risk in patients with persistent normoalbuminuria (1). Since 47 percent of patients with diabetic nephropathy were not able to perform a conclusive exercise-ECG test, we extended the cardiac examinations with cardiovascular magnetic resonance imaging (CMR). We used CMR to assess left ventricular, mass, and dimensions and to estimate the prevalence of plaque burden and silent coronary heart disease. It is well validated that CMR allows for non-invasive detection of coronary artery stenosis (38) and imaging of atherothrombosis in the aorta (39;40), the carotid (41) and coronary arteries (42-44). CMR is much more accurate than echocardiography and is considered the gold standard for evaluation of left ventricular function and anatomy (45). CMR has unsurpassed accuracy and reproducibility for estimation of left ventricular function, volumes, and mass due to its excellent image quality and three-dimensional coverage which precludes geometrical assumption (46). The method is non-invasive and thus with no risk for the patient and there was no need for contrast agents. This was of huge importance since the patients where free of symptoms or signs of heart disease and thus a risk for the individual was not acceptable. Invasive x-ray angiography carries a risk of a serious adverse event (death or severe neurological event) of approximately 2 ‰ (47). The assessment of coronary artery stenosis was based on visual inspection of the coronary angiograms using a previously validated approach with a sensitivity for detection of significant coronary stenosis (compared to quantitative x-ray angiography) of 88-93% (48).
By using CMR we had the possibility to assess plaque burden, which is not possible by x-ray angiography. Right coronary artery (RCA) vessel wall scanning for evaluation of plaque burden was done in a subset of subjects when total scan time did not exceed one and a half hour (24 with and 37 patients without nephropathy, respectively) using 3D black-blood imaging according to a previously validated protocol (44;49). Plaque burden can also be assessed by intravascular ultrasound (IVUS), however we did not wish to use an invasive technique in our patient population due to risk for the individual patient for a serious adverse event.
Computer Tomography (CT) can also be used for evaluation of left ventricular parameters (50;51) and for assessment of coronary stenosis (52-55). However patients receive radiation, and also nephrotoxic contrast material, which is not used in a CMR scan. Since many of our patients had a low kidney function we found CMR to be preferable, since the information and data from CMR and CT is comparable (56).

4.5 Peripheral artery disease:

Peripheral artery disease (PAD) was in patients without lower limb amputation evaluated by measurement of the systolic blood pressure in the big toe by a strain gauge technique (57). Severe PAD was considered evident in patients with a history of amputation due to ischaemia, of claudicatio intermittens, or a systolic blood pressure in the big toe ≤ 30 mmHg (58).

4.6 Laboratory analysis:

Markers of low-grade inflammation and endothelial dysfunction and transforming growth factor-beta (TGF- ß):
Analyses of the biomarkers of endothelial dysfunction and low grade inflammation were performed at a central lab in Amsterdam by Casper Schalkwijk. We measured C-reactive protein (CRP) with highly sensitive in-house sandwich enzyme immunoassays. Rabbit antihuman CRP immunoglobulins were used as catching antibodies; peroxidase-conjugated rabbit antihuman CRP immunoglobulins were used as detecting antibodies (Dako, Copenhagen, Denmark). o- phenylenediamine (Sigma Chemical Co., St Louis, MO, USA) acted as substrate for CRP antigen. The intra- and interassay coefficients of variation were 3.9% and 8.7% for CRP.
We measured plasma levels of soluble (s) vascular cell adhesion molecule-1 (VCAM-1; Diaclone, Besanc¸on, France) (range for assay 538 - 1286 ng/mL), soluble intercellular adhesion molecule-1 (ICAM-1; Diaclone)) (range 98 - 647 ng/mL), and plasminogen activator inhibitor-1 (PAI-1) antigen (Innogenetics, Gent, Belgium) in duplicate by use of commercially available enzyme-linked immunosorbent assay (ELISA) kits. The intra- and interassay coefficients of variation were 4.4% and 4.6% for sVCAM-1; 4.0% and 7.4% for sICAM-1; and 2.8% and 8.2% for PAI-1.
Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used for measurements of interleukin-6 (IL-6), secreted phospholipase A(2) (sPLA2), and plasminogen activator inhibitor-1 (PAI-1). (Quantikine High Sensitivity; R&D Systems, U.K.).
Total TGF-ß was measured by an ELISA Development system (R&D Systems).
The laboratory analysis of the biomarkers was done on freezer (-80Ċ) samples stored since baseline examination in 1993 and analyzed in 2002.

N-terminal-pro-brain natriuretic protein (NT-proBNP):
Blood for determination of NT-proBNP was taken after the patients had been at rest for at least 20 min in the supine position, blood samples was centrifuged and plasma stored at −80°C until analysis. Plasma concentrations of NT-proBNP were measured by a sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics, Basel, Switzerland). The intra-assay variation is below 3.0% and the total coefficient of variation ranges from 2.2% to 5.8% in low and high ranges of NT-proBNP.

Pregnancy associated plasma protein-A (PAPP-A):
Fasting blood samples were taken and blood was centrifuged within 1 hour and plasma was placed into aliquots and stored at -80°C until analysis. PAPP-A levels were determined by means of a biotin-tyramine-amplified enzyme immunoassay with a limit detection of 0.03 mIU/L. All samples were determined within the assay measuring range.
PAPP-A polyclonal antibodies were used for capture, and a combination of monoclonal antibodies were used for detection. The assay was calibrated against the World Health Organisation´s international reference standard 78/610, which is standard for pregnancy-associated proteins. Levels of eosinophil major basic protein (proMBP), the endogenous inhibitor of PAPP-A were determined by means of an immunoassay developed at Statens Serum Institute, Copenhagen. Within the calibrator range used, the intraassay variation was <5% (59)

Placental growth factor (PlGF):
After the patients had been at rest for at least 20 minutes in the supine position, blood samples for determination of PlGF were collected, centrifuged and the plasma stored at -80 °C until meas-

urement. Plasma concentrations of PlGF were measured by an enzyme linked immunosorbent assay (R&D, Wiesbaden, Germany). The intra-assay variation is below 10 % and the total coefficient of variation ranges between 3.6 % and 11.8 % in low, medium, and high ranges of PlGF.

KIDNEY DISEASE IN DIABETES MELLITUS: INCIDENCE AND PROGNOSIS

Decreased incidence of diabetic nephropathy

Today, treatment and clinical care of patients with diabetes is mainly focused on preventing the development of late diabetic complications. For many years the prevention and postponing of diabetic complications has been a major area of research. The most devastating diabetic complication is the development of diabetic nephropathy. New treatment modalities proved to decrease incidence of persistent proteinuria in patients with type 1 diabetes. Patients with onset of diabetes in the sixties had a lower incidence of persistent proteinuria than patients with onset of diabetes in the thirties (14-16). However even though the incidence was reported to have declined, still 25% of type 1 diabetic patients developed diabetic nephropathy after 25 years of diabetes duration. A Swedish study reported in 1994 a very low incidence of diabetic nephropathy (5.8% after 20 years) (60), however this could not be found in a Danish cohort (61) where the incidence of diabetic nephropathy was 16 % after 15 years in patients with onset of diabetes between 1975-1979.

In 2003 Hovind et al (17) reported a declining incidence of diabetic nephropathy. In type 1 diabetic patients a declining incidence from 31.1% in patients with debut of diabetes from 1965-1969 falling to 13.7% in patients with debut from 1979-1984 was shown (data restricted to those patients with 20 years of follow-up).

In patients with type 2 diabetes similar observational data is not available, but among Pima Indians the incidence of diabetic nephropathy is reported in 2006 to be increasing due to an increase in the proportion of patients with long diabetes duration (62). However the incidence of ESRD was falling (62) in the Pima Indians probably due to better treatment of glycaemic control, blood pressure, and dyslipidaemia. In a Danish study it was recently shown that the incidence of patients with type 2 diabetes referred to renal replacement therapy is stabilized (63) and a Finnish study has found the same in type 1 diabetic patients (64). Furthermore the survival in both type 1 and type 2 diabetic patients with ESRD has improved and patients are in general older at the time of referral for treatment of ESRD (65). Most likely this is a result of intensive renoprotective treatment in patients with diabetic nephropathy and in patients with microalbuminuria, but also better glycaemic control and treatment of dyslipidaemia and hypertension in all patients with diabetes.

IMPROVED PROGNOSIS IN PATIENTS WITH DIABETIC NEPHROPATHY

The recognition that treatment of hypertension altered the natural way of progression of diabetic nephropathy (20;66) has led to improved survival and better life quality among type 1 diabetic patients with diabetic nephropathy (1;67). In early days the median survival of type 1 diabetic patients with diabetic nephropathy after onset of persistent proteinuria was 5-7 years (68;69). At that time insulin was the sole treatment for patients with diabetes, and thus no antihypertensive treatment, treatment of ESRD with renal transplant or dialysis was given. The primary cause of death was ESRD (66% of the patients) (14). Patients died from uraemia after a long phase with extreme sickness, unbearable itch, and severe oedema. The discovery that antihypertensive treatment could reduce albuminuria and reduce the decline in kidney function in type 1 diabetic patients with persistent albuminuria (70), was the first step towards improved treatment possibilities and improved survival in patients who developed diabetic nephropathy (20;66;71;72).

The prognosis in patients developing persistent proteinuria was changed with the antihypertensive treatment. Parving and Hommel showed in 1989, that prognosis was improved when patients with diabetic nephropathy and diastolic blood pressure above 95 mmHg was treated with antihypertensives - in these patients the 10 year mortality was 18% (73) and the median survival 16 years (74). In 1989 Mathiesen et al also demonstrated a large reduction in mortality after introduction of antihypertensive treatment (75). In 1996 Rossing et al showed data from a cohort started in 1984 and followed for 10 years (67). The main focus of their study was to identify predictors of mortality in type 1 diabetic patients. Two-hundred-sixty-three patients were followed hereof 165 patients with diabetic nephropathy. The median survival after onset of diabetic nephropathy in this cohort was 14 years compared to a median survival of 5 years in data presented by Andersen et al in 1983 (14). Also a decline in cause of death from ESRD was found between the studies; 35% compared to 66%.

Ten years later we sought to evaluate if prognosis had improved even further in a cohort started in 1993 at the Steno Diabetes Center (12;76), including 401 patients with or without diabetic nephropathy. At this time arterial hypertension was defined and treated according to the World Health Organization's criteria (≥165/95 mmHg) (77). After the results from the captopril collaborative study in 1993 (78) inhibitors of the angiotensin converting enzyme was recommended as the initial step in treatment of diabetic nephropathy in type 1 diabetic patients at the Steno Diabetes Center. After the results from the UKPDS (79) guidelines was changed at Steno Diabetes Center, and antihypertensive treatment was recommended to all patients with blood pressure ≥140/90 mmHg from 1999 (80;81).

From 2002 all type 1 diabetic patients with diabetic nephropathy and all patients with type 2 diabetes was recommended aspirin and statins.

In conclusion, our study (1) confirmed and exceeded the major improvement in survival in type 1 diabetic patients with diabetic nephropathy. The estimate of median survival from onset of diabetic nephropathy was 21.7 years, standard error 3.3 years. Patients with overt diabetic nephropathy at baseline were left truncated and contributed from the time corresponding to duration of overt nephropathy at the baseline examination. In order to make our data comparable with historic data (14;67;69;74), we made a secondary analysis combining death and renal death (patients alive and treated with dialysis or renal transplantation, since these patients in the past would have died from ESRD). In this analysis the median time until ESRD or death was 18.3 years, SE 2.1 years. In figure 1 our survival estimate, including renal death, is compared with historic data (14;67;69;74).

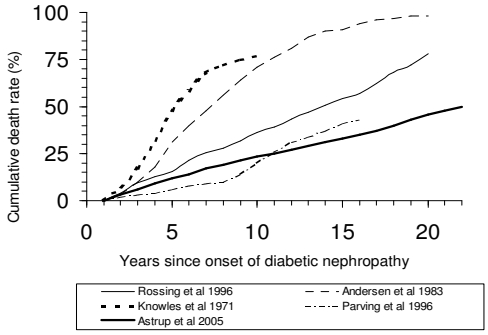

Figure 1: Cumulative death rate (including renal death) in type 1 diabetic patients after onset of diabetic nephropathy. Figure from reference (1).

The improved prognosis in our cohort of patients with diabetic nephropathy was mostly due to aggressive long term antihypertensive treatment in addition to improved glycaemic control and smoking cessation, since treatment with statins and low-dose aspirin were not implemented until the near end of follow-up. However, even though we found an improvement in prognosis, compared to previous studies, we found that during follow-up 40% of all patients with diabetic nephropathy had experienced a combined endpoint of cardiovascular mortality and morbidity, combining cardiovascular death, history of nonfatal myocardial infarction, percutanous coronary intervention (PCI), coronary artery bypass grafting (CABG), nonfatal stroke, amputation as a result of ischemia, and vascular surgery for peripheral atherosclerotic disease (PAD). This was in contrast to patients with persistent normoalbuminuria where only 10% experienced an endpoint during 10 years of follow-up. Thus diabetic nephropathy remains a major risk factor for cardiovascular morbidity and mortality in type 1 diabetic patients.

In our study (1) we evaluated known risk factors among survivors participating in the follow-up examination and compared the data to the baseline data in the same patients. Glycaemic control had improved over time, fewer patients smoked, blood pressures were lower, and total cholesterol, LDL-cholesterol was decreased, and HDL-cholesterol was increased. The change in cholesterol fits the recommendation of statins to all patients with diabetic nephropathy, however only 50% of patients with diabetic nephropathy were in treatment with statins at follow-up, and 65% of patients received aspirin. One week before the baseline examination patients were asked to stop their antihypertensive treatment, and 66% of patients did, so blood pressure values at baseline cannot be directly compared to blood pressures at follow-up. However the treatment with antihypertensives was much more intensive at the follow-up examination and here all patients (except two patients in dialysis) received antihypertensive treatment predominantly with blockade of the renin-angiotensin-system. Even though cardiovascular risk factors improved in survivors during follow-up many of the patients who died did not receive the more intensive treatment of cardiovascular risk factors. The beneficial change in cholesterol levels has most likely occurred late in follow-up, whereas the more aggressive treatment of blood pressure was implemented earlier during follow-up. Thus prognosis might improve even further with better glycaemic control and treatment with statins and low-dose aspirin and more aggressive treatment with antihypertensives from onset of diabetic nephropathy or when patients develop microalbuminuria. Consequently, our study (1) indicates that the future focus of patient care must be multifactorial treatment aiming at postponing ESRD and reducing cardiovascular disease. This has already been shown to have improved prognosis in patients with type 2 diabetes and microalbuminuria in the Steno 2 follow-up trial (82).

MULTIFACTORIAL AGGRESSIVE TREATMENT AND LIFETIME RISK

The prognosis in patients with diabetes has improved with the possibility to treat blood pressure, dyslipidaemia, hyperglycaemia, and use of antithrombotic agents. Patients today still suffer from renal disease, however the life expectancy after onset of diabetic nephropathy has increased substantially, and many patients with diabetic nephropathy today die from cardiovascular disease. The aim of treatment is now to prevent or postpone late diabetic complications, including cardiovascular disease. Patients with type 2 diabetes have a two to six times higher risk of death from cardiovascular causes than individuals without diabetes (83;84). Among patients with type 1 diabetes a similar increased risk is seen, and the reason is mainly the very high risk of developing cardiovascular disease among patients with diabetic nephropathy who have a 37-fold relative risk increase of death from cardiovascular disease as compared to the background population (85).

It is well known that intervention towards modifiable risk factors such as hypertension, hyperglycaemia, and dyslipidaemia is beneficial, however most studies have evaluated intervention towards one risk factor at a time. Intensive treatment of blood glucose has proven beneficial in the Diabetes Control and Complications Trial (DCCT) in patients with type 1 diabetes (86) and risk of developing nephropathy, neuropathy, and retinopathy was reduced with decreasing values of HbA1c (87). In type 2 diabetes the UKPDS study showed better outcome with better glycaemic control (88;89). However there might be a limit to how strict glycaemic control should be, at least this seems to be the case in patients with type 2 diabetes. Recently the intensive glucose arm of the ACCORD study was stopped due to a higher mortality among patients in the intensively treated group. Patients randomized to having their HbA1c treated to a target of 6 (the mean value came down to 6.4) had a higher all-cause mortality, but fewer non-fatal myocardial infarctions, than patients in the less stringent treated group (here mean HbA1c was 7.5) (90). The HbA1c was lowered during short time, and this might not be beneficial with more episodes of hypoglycaemia. Also adverse effects to the medication and weight gain might be an explanation. In the ADVANCE study and in the VADT intensive glycaemic control also failed to reduce cardiovascular outcomes in patients with type 2 diabetes (91;92). These large trials still leave debate on treatment goals regarding glycaemic control. A recent position statement concludes on basis of these large trials, that goals of treatment are not so black and white, rather treatment goals should be individualized. Thus some patients might benefit from near to normal HbA1c values, and some patients (i.e. patients with known CVD) should have less stringent HbA1c levels (93).

Antiplatelet therapy is known to reduce risk of serious vascular events (94), and arterial occlusion (95) and venous thrombosis (96) among patients at high risk of cardiovascular disease, but also in patients with stable angina and peripheral arterial disease (97). Further more low dose aspirin is an effective antiplatelet regimen for long-term use with little side effects (97) making it suitable for lifetime intervention in patients with diabetes - but the effect in patients with diabetes seems to be less than in non-diabetic pa-

tients (97;98). The reason for this is not known and the phenomenon is called aspirin resistance and the mechanisms is recently debated by Ajjan et al (99). Thus patients with diabetes might not have the same effect from aspirin as non-diabetics. Recently two prospective, placebo-controlled randomized studies failed to show a benefit from aspirin treatment on hard endpoints (100;101). In the metaanalysis performed by the antithrombotic trialists' collaboration the subgroup of patients with diabetes had a 7% risk reduction, however this was not significant (97).
In the Heart Protection Study (HPS) direct evidence that cholesterol-lowering therapy is beneficial in patients with diabetes that not already had manifest cardiovascular disease was presented. This was prove of concept for prophylactic treatment with statins in all patients with type 2 diabetes. A subgroup of 615 patients (10%) had type 1 diabetes and the trend towards benefit was seen but the numbers were too low to reach statistical significance. In the West of Scotland follow-up study the importance of early intervention was proved in regards to statins. Two groups of men with hypercholesterolemia with no history of myocardial infarction were randomized to either placebo or treatment with statins. A benefit was seen after 5 years of follow-up (102), however the importance of early intervention was evident after the sustained follow-up. Here an ongoing reduction in risk of major coronary events among study participants treated with pravastatin during the trial was seen even though equally many in both groups received statins in the sustained follow-up period (103).
In the United Kingdom Prospective Diabetes Study (UKPDS) tight blood pressure control was shown to decrease the risk of developing microvascular and macrovascular complications in patients with type 2 diabetes (79). The Steno-2 trial (21) went further and was the first study to evaluate multifactorial intervention. Subjects with type 2 diabetes and microalbuminuria were randomized to routine treatment at their own general practitioner, or intensive treatment lifted by a specialized diabetes team including doctors, nurses, and dieticians. Patients in the intensive treatment group received lifestyle intervention, statins, aspirins, aggressive antihypertensive treatment, and guidance in obtaining improved glycaemic control. Cardiovascular outcome was evaluated after approximately 8 years of follow-up and benefit of multifactorial intervention was seen as a decreased risk of cardiovascular disease, decreased risk of developing diabetic nephropathy and retinopathy, and less severity of autonomic neuropathy (104). The benefit of being in the initial intensively treated group was further improved after 13 years of follow-up with a nearly 50 percent risk reduction on all-cause mortality, even though all patients after 8 years was offered the same treatment in routine outpatient clinic (82). These results underline the importance of early aggressive multifactorial intervention in patients with a high lifetime risk.
Today multifactorial treatment is considered intervention as it was done in the Steno-2 trial. However new areas of treatment is coming up, and it might be that in the future multifactorial treatment includes many more drugs: AGE-breakers (ongoing trial in Type 1 diabetic patients), and erythropoietin for patients with anaemia caused by diabetic kidney disease (ongoing Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) in type 2 diabetic patients). Anti-vascular endothelial growth factor (VEGF) has been proved beneficial in the treatment of diabetic eye disease (105). As described later chronotherapy might also prove beneficial in the treatment in hypertensive subjects with an abnormal circadian rhythm of blood pressure. Inflammation and endothelial dysfunction are known to play a role in the development of cardiovascular disease (106-109), and a recent post hoc analysis of the IRMA-2 trial showed that irbesartan reduces inflammatory activity in patients with type 2 diabetes and microalbuminuria and actually reduced progression of diabetic nephropathy (110).
Most studies evaluating prognosis and improvement after initiation of a specific intervention concludes on the basis of 5 to 10 years of follow-up. Risk scores developed to guide clinicians with treatment decisions are mostly describing 10 year risk. However, patients with both type 1 and type 2 diabetes might be young and thus have a small 10 year risk, even though their lifetime risk is very high. Therefore it is likely that aggressive multifactorial intervention might have the most benefit if started early when the 10 year risk is still low. The cascade leading to cardiovascular disease in these individuals might be started much before the diabetic kidney disease becomes evident, and thus intervention should also be started early to prevent the process of angiopathy to start. This might reduce lifetime risk.

PREDICTION OF CARDIOVASCULAR RISK IN DIABETES-

Physiological risk factors in type 1 and type 2 diabetes:

Hypertension – central blood pressure – and dipping:
Diabetes and hypertension are often associated, and both conditions increase risk of cardiovascular disease and renal disease (111-113). Lowering of blood pressure reduces risk in the general population (114) and in type 1 diabetic patients antihypertensive treatment has improved prognosis in patients with diabetic nephropathy (20;66). In type 2 diabetic patients the UKPDS showed a better prognosis with more tight blood pressure control in regards to both microvascular and macrovascular complications to diabetes (79). The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) was the first trial to compare "older" to "newer" drugs (beta-blockers with addition of thiazides vs. calcium channel blockers with addition of ACE-inhibitors). Even though the reduction in brachial blood pressure were similar, the "newer" drugs showed a larger reduction in cardiovascular disease and death (115). A part of the explanation can perhaps be found in the sub-study of ASCOT, the Conduit Artery Function Evaluation (CAFE) trial where the "newer" drugs was found to reduce central aortic blood pressure more than the "older" drugs (116). This reduction in central aortic blood pressure was significantly associated with a reduction in the composite endpoint of cardiovascular event/procedures.
The routine way to measure blood pressure is by office brachial pressure, however as shown clearly in the CAFE trial brachial blood pressure might not be the most informative way of measuring blood pressure. Studies evaluating the impact of blood pressure as a risk factor for mortality have been based on few blood pressure measurements in a clinical setting. Twenty-four hour ambulatory blood pressure has been shown previously to be superior to office blood pressure in terms of monitoring of hypertension, and in predicting cardiovascular risk in patients with essential hypertension (117;118) and in the general population when assessing the cardiovascular risk and all-cause mortality (119;120), and when evaluating the progression of microvascular complications in diabetic patients (121). Furthermore, there is a higher reproducibility of 24 h ambulatory blood pressure compared to office blood pressure, and in addition information about white coat hypertension is obtained. Also information about the circadian rhythm of the blood pressure is obtained when 24 h ambulatory blood pressure is measured, and night blood pressure has been shown to be superior to day blood pressures in predict-

ing cardiovascular risk and risk of death in the general population (120). Lack of the nocturnal decline in blood pressure has been associated with LVH and with future cardiovascular events in hypertensive women with essential hypertension (122). It has also been suggested that abnormal circadian variability in blood pressure predicts the development of microalbuminuria in patients with Type 1 diabetes (123). Limited information is available regarding the long term prognostic impact on mortality of 24 h ambulatory blood pressure compared to office blood pressure and abnormal circadian blood pressure rhythm in type 2 diabetic patients, as this has only been assessed in a study of a mixture of both type 1 and type 2 diabetic patients (124) or in Japanese type 2 diabetic patients (125). We therefore conducted a prospective study in type 2 diabetic patients to evaluate the impact of abnormal circadian rhythm of blood pressure as verified from the 24 h ambulatory blood pressure measurements (8).

In a thirteen years follow-up study in type 2 diabetic patients with and without diabetic nephropathy we found patients with abnormal circadian blood pressure, based on one single determination, to have a higher mortality than patients with a normal dipping profile. Non-dipping of night blood pressure predicted all-cause mortality even after adjustment for traditional cardiovascular risk factors. In addition to the important information about circadian blood pressure rhythm the ambulatory blood pressure was a better predictor of mortality than office blood pressure in our study (8). This is in accordance with the follow-up results from the Pressioni Arteriose Monitorate e Loro Associazioni (PAMELA) study where risk of death increased more with a given increase in ambulatory blood pressure than in office blood pressure in the general population (126). If blood pressure was higher during the night than during day (reversed pattern) the prognosis was found to be even worse (figure 2), similar results was found in the Japanese study (125).

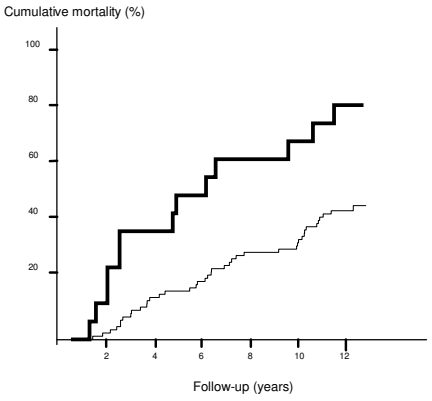

Figure 2:
Type 2 diabetic patients with reversed pattern (thick line) of blood pressure (n=16), higher blood pressure in night time than in day time, compared to patients with normal or reduced dipping (thin line) of blood pressure (n=88). Log rank test for differences between groups p=0.001. Figure from reference (8)

The physiological explanation for non-dipping has been debated (127) and a possible explanation might be a lack of peripheral vasodilatation during night time due to sustained adrenergic activity (128). In sympathectomised patients there is no dipping of night blood pressure. Also severe autonomic neuropathy may impair the circadian blood pressure rhythm (129;130). Sleep apnea is known to cause increased nocturnal blood pressure due to enhanced cardiac pre-load and hypoxia and this could be an explanation to the non-dipping phenomenon in some patients. Hypoxia elicits increased levels of norepinephrine, endothelin and erythropoietin (131) and thus causes blood pressure to increase. Potentially beta-blockers could be of benefit in this patient group to correct these mechanisms.

Most patients with type 2 diabetes are treated with antihypertensive medication, and the importance of the nocturnal blood pressure load demonstrated in this study, suggests that full 24 h effect of the antihypertensive medication is very important. Chronotherapy of hypertension provides a possibility to optimize the circadian blood pressure profile in the individual patient according to the 24 h ambulatory blood pressure (132). If non-dipping marks a patophysiological process that in it self is responsible for the higher mortality, one should not expect a large benefit of correcting just the symptom (non-dipping) rather than interfering with the process of non-dipping. We found non-dipping to increase mortality in type 2 diabetic patients even after adjusting for 24 h ambulatory blood pressure, suggesting that the non-dipping per se is dangerous beyond the overall higher blood pressure. The Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) did not find a risk reduction in patients treated with telmisartan (longer acting) compared to patients treated with ramipril (shorter acting) (133). However the patients in the study were selected among patients already with coronary, peripheral, or cerebrovascular disease and the patients were not characterized as dippers or non-dippers. It might be that the more smooth 24 h blood pressure control given by telmisartan compared to ramipril alone could have a beneficial effect in patients with non-dipping of night blood pressure if treatment were initiated at an earlier state. Recommendations in literature to target non-dipping is a long acting blood pressure lowering agent, i.e. telmisartan and/or amlodipin, or to split dose between morning and evening to provide a more smooth effect (134).

In conclusion we found type 2 diabetes patients with non-dipping of night blood pressure to be at higher risk of death as compared to dippers independent of known cardiovascular risk factors. Since non-dipping has a high prevalence among patients with diabetic nephropathy measurement of 24 h ambulatory blood pressure should be used in order to assess full risk profile and effect of blood pressure lowering therapy in this patient group. The next step in management of the non-dipping phenomenon must be to do a trial where the therapeutic response to chronotherapy is evaluated by systematic night-time blood pressure recording in non-dippers. The optimal trial would evaluate long-term effect of a positive treatment response.

In type 1 diabetic patients we have in a long-term prospective follow-up study (1) with 391 patients shown systolic blood pressure to be a predictor of the combined endpoint of cardiovascular mortality and morbidity. The follow-up study was conducted in order to evaluate prognosis in type diabetic patients with and without diabetic nephropathy and to evaluate predictors of outcome. Systolic blood pressure was found together with other strong risk factors (age, history of previous event, and presence of nephropathy) to predict outcome. In early days when blood pressure was less aggressively treated systolic blood pressure was a very strong determinant of outcome. In our follow-up study we still found systolic blood pressure to be a predictor of outcome even though blood pressure was treated aggressively. Therefore it might be there is still some potential benefit for the patients if blood pressure is treated even more aggressively.

CARDIAC AUTONOMIC NEUROPATHY

Cardiac autonomic neuropathy (CAN) is a severe complication of diabetes, causing death and morbidity, and large costs to the welfare system (135).
CAN results from damage to the autonomic nerve fibers to the heart, and the earliest indicator of CAN is a decrease in heart rate variation (HRV) during deep breathing (136), which is easily assessed by a simple bedside test. Originally the association between CAN and poor prognosis was proposed by Ewing (137). In this early study risk factors such as nephropathy and known CVD were not assessed, and it is likely that these factors contributed importantly to the increase in mortality in the patients with CAN. In a meta-analysis of 12 published studies abnormal HRV was shown to be associated with an increased risk of silent myocardial infarction (33) and in 1123 patients with type 2 diabetes CAN was a strong predictor of cardiac ischemia.
Prospective studies have demonstrated increased mortality in patients with CAN (138-140). Rathmann et al (138) investigated a mixture of type 1 and type 2 diabetic patients, with a total of 35 patients with CAN, and found an 8 year survival rate of 77% in these patients. Ewing et al (139) showed an increase in sudden death in patients with autonomic neuropathy with 8 sudden deaths among 71 diabetic males followed for 3 years. In a larger study population of 457 type 1 diabetics Orchard et al (140) found an 4-fold increase in mortality in patients with CAN after two years of follow-up.
Patients who develop diabetic nephropathy carry a higher risk of all-cause mortality and CVD (1), and since CAN is known to be associated with cardiovascular disease we conducted a prospective observational follow-up study to evaluate the prognostic value of CAN in a large cohort of type 1 diabetic patients with and without diabetic nephropathy (3). The cohort was followed prospectively for 10 years and the prognostic value of HRV in relation to all-cause mortality, and a combined endpoint of cardiovascular morbidity and mortality and to progression of diabetic nephropathy was assessed. We found autonomic dysfunction to be an independent predictor of cardiovascular mortality and morbidity in patients with diabetic nephropathy (3). In patients with persistent normoalbuminuria individuals with abnormal HRV had a higher incidence of CVD than individuals with a normal HRV (figure 3); however the significance of results was attenuated after adjustment for traditional risk factors.
In type 2 diabetic patients, we had the opportunity to evaluate the prognostic importance of HRV in a long-term prospective study designed to evaluate predictors of mortality in type 2 diabetes (8). The follow-up time was 13 years and type 2 patients with or without diabetic nephropathy was enrolled in a case control study. We found HRV was a significant predictor of all-cause mortality even after adjustment for other strong cardiovascular risk factors (8).
Autonomic dysfunction rarely exists as an isolated complication in long-term diabetes (33). Often coexistence with coronary artery disease, cerebrovascular disease and nephropathy is seen (33). It has been suggested that the development of CAN and deterioration of UAER occurs simultaneously (141), however we did not find a relationship between rate of decline in glomerular filtration rate and abnormal HRV in our study with type 1 diabetic patients with diabetic nephropathy.
The mechanisms by which CAN exerts negative influence on quality and length of life is controversial. One hypothesis involves impaired central control of respiration in patients with CAN (142). Many relations have been made i.e. to exercise intolerance (143-145), with reduced response in heart rate and blood pressure, to decreased cardiac output during exercise, to silent myocardial ischemia (33;138;146-148), and to prolongation of QT interval causing deadly arrhythmias (149-152).
Treatment and prevention of CAN have not received much focus and prospective trials evaluating benefits on outcome with treatment of CAN is missing. However, in the DCCT study good glycaemic control was shown to slow progression of abnormal autonomic tests (153), and as recently reviewed symptomatic treatment of peripheral neuropathy is possible with ACE inhibitors and beta blockers (31). Furthermore a short term study showed an increase in HRV during treatment with an ACE-i (154). In the Steno-2 study it was shown that patients with type 2 diabetes and microalbuminuria developed less autonomic neuropathy when treated aggressively with multifactorial treatment (104). A review article by Maser et al (155) suggest to treat CAN and monitor treatment with yearly measurements of HRV. However, intervention towards abnormal HRV has not been proven beneficial in hard endpoint studies yet but treatment of CAN may provide new aims and possibilities for multifactorial treatment.

Figure 3a:

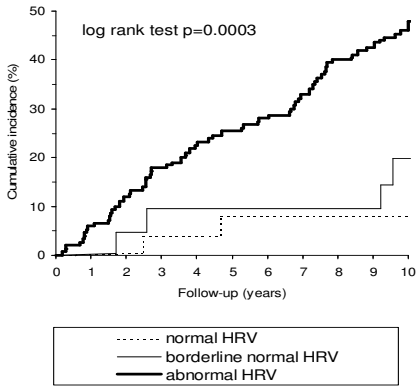

Figure 3b:

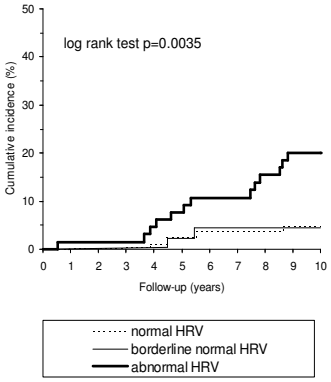

Figure 3a and 3b: The cumulative incidence of the primary endpoint in patients with diabetic nephropathy (a) and in patients with persistent normoalbuminuria (b) with patients divided into categories as suggested by Ewing (35).

LEFT VENTRICULAR FUNCTION AND VOLUMES AND RELATION TO AUTONOMIC NEUROPATHY

In 1972, Rubler et al suggested for the first time the existence of diabetic cardiomyopathy as a distinct entity (156). The hypothesis was that diabetes mellitus affects cardiac structure and function potentially leading to heart failure. Characteristic histological changes with myocellular hypertrophy, thickening of the capillary basement membranes, lipid disposition, interstitial fibrosis can be observed as reviewed by Korosoglou and Humpert (157). Hyperglycaemia and increased non-enzymatic glycation might be the explanation for these structural changes leading to impaired ventricular function in patients with diabetes mellitus (158). In the Multi-Ethnic Study of Atherosclerosis (MESA) diabetes was shown to impair left ventricular function independent of coronary atherosclerosis (159).This impairment of left ventricular function seems to ad to the increased cardiovascular risk caused by coronary atherosclerosis in patients with diabetes. In the Diabetes Insulin Glucose in Acute Myocardial Infarction (DIGAMI) study in patients with diabetes mellitus suffering from myocardial infarction the most common cause of death (66%) in the first year following myocardial infarction (160) was heart failure. Patients with diabetic nephropathy have an increased risk of cardiovascular disease (1) and often cardiovascular disease is diagnosed late in these patients. With the improved prognosis, and the shift in causes of death towards more cardiovascular disease rather than kidney disease, the importance of diagnosing and intervening towards cardiovascular disease becomes an increasing clinical challenge in managing the patient with long-term diabetes and especially the patient with diabetic nephropathy. Therefore we conducted a study to evaluate the effect of diabetic nephropathy on atherosclerosis and left ventricular mass, function, and volumes. We enrolled 136 patients with type 1 diabetes hereof 63 patients with diabetic nephropathy and 73 patients with persistent normoalbuminuria in a case control study (7). All patients were without history or symptoms of heart disease. We used CMR to evaluate subclinical coronary and aortic atherosclerosis and left ventricular mass, function, and volumes. In this section function and volumes and relation to other clinical measures is discussed. Our study (7) is the first study to evaluate left ventricular mass, function, and volume with CMR in a large cohort of asymptomatic patients with long-standing type 1 diabetes. We demonstrated normal global left ventricular systolic function and normal filling pressures in all patients in accordance with their clinical status. Our data match results from the Framingham Heart Study (45) where an adult population free of hypertension underwent CMR. In our study, patients with nephropathy and without known CVD had considerably lower systolic blood pressure and diastolic blood pressure compared to earlier studies (161) due to aggressive antihypertensive treatment and would be less likely to have LVH and impaired left ventricular function. Therefore, our study reflects cardiovascular function in asymptomatic type 1 diabetics on contemporary reno- and cardio protective medication (162). Surprisingly, we found that patients with nephropathy had smaller left ventricular volumes compared to normoalbuminuric patients. One explanation for this might be that patients with diabetic nephropathy had more autonomic neuropathy and higher levels of HbA1c. This is based on the finding of a negative correlation between HbA1c and LVM and volumes showing that patients with high levels of HbA1c had smaller LVM and smaller left ventricular volumes. A possible explanation is that patients with a higher HbA1c tend to have a higher degree of autonomic neuropathy (3;33) resulting in a relatively higher heart rate with smaller left ventricular dimensions as a consequence to keep the same cardiac output. In accordance with that, our data showed a higher HbA1c was correlated to smaller heart rate variability and patients with high levels of HbA1c had an increased heart rate with smaller left ventricular dimensions. Thus, autonomic neuropathy in our normotensive population seems to induce negative left ventricular remodeling, particularly in patients with diabetic nephropathy, since these patients in general have poorer glycaemic control and more severe autonomic neuropathy compared to patients with normoalbuminuria.

LIFESTYLE

Sedentary lifestyle is major problem in the western world today and in part this is causing the increasing number of patients with type 2 diabetes. Studies as the Steno-2 trial tried to address the risk factor “lifestyle” with guidance of patients in the intensively treated arm of the study to stop smoking, change their diet, and increase exercise (104). Cessation of smoking is well known to reduce cardiovascular risk and smoking remains the number one preventable cause of morbidity and mortality in the United States (163). Studies on the effect of smoking on progression in kidney disease have come out with contradicting results. Some studies find a more rapid decline in kidney function in smokers (164-166) and others report no differences between smokers and non-smokers (167). Most of the risk factors for developing micro- and macrovascular disease are the same in diabetic patients, and clearly the increased risk of macrovascular disease in smokers is beyond doubt. Smoking as a risk factor for cardiovascular mortality and morbidity in patients with chronic renal disease has recently been reviewed in detail by Orth (168).
Other components of lifestyle change have been difficult to prove beneficial per se, since the intervention is difficult to carry out. In the Steno-2 study patients randomized to intensive therapy received expert help to change their diet, they were recommended exercise for at least 30 minutes three to five times a week and patients who were smokers were invited to smoking cessation courses. However after follow-up the only significant change between groups were in relative intake of fat and carbohydrates (104). BMI, amount of exercise, and number of smokers did not change between groups despite of a large effort from a trained staff. Thus the beneficial effect of being in the group with intensive therapy was mainly ascribed to lower blood pressure, better glycaemic control, and improved lipid profile - all parameters that were targeted with medicine. However this should not undermine the importance of a healthy lifestyle.

MORPHOLOGICAL RISK FACTORS

Plaque burden and subclinical coronary artery disease

Coronary heart disease is a major cause of mortality and morbidity in both type 1 and type 2 diabetes (169). Heart disease if often diagnosed late in patients with diabetes and lesions are more pronounced at the time the patient is diagnosed, perhaps as a consequence of silent heart disease in patients with diabetes (33;138;146-148). We have shown that patients with type 1 diabetes and diabetic nephropathy have a very high risk of developing cardiovascular disease over a period of ten years compared to patients with persistent normoalbuminuria (1), and consequently

we wished to examine similar patients, still asymptomatic, in order to evaluate silent cardiovascular disease, plaque burden, and coronary heart disease (6).
Therefore we conducted a cross-sectional study to evaluate the effect of diabetic nephropathy on atherosclerosis in type 1 diabetes using CMR to evaluate subclinical coronary and aortic atherosclerosis in asymptomatic type 1 diabetic patients with and without nephropathy (6). All patients also had an exercise-ECG performed however this examination was not very suitable for risk assessment in patients with diabetic nephropathy since only 47% was unable to achieve 80% of their maximum predicted heart rate during the test, resulting in inconclusive test results. With CMR we found a greater coronary plaque burden and a higher prevalence of coronary artery stenosis in patients with diabetic nephropathy compared to those with normoalbuminuria. Actually patients with normoalbuminuria had a low prevalence of coronary plaques (15%), and none had coronary artery stenosis or ischemia on exercise testing. This is in good agreement with the relative low risk of cardiovascular mortality and morbidity in patients with persistent normoalbuminuria we found in the 10 year follow-up study (1).
Among patients with diabetic nephropathy 76% had coronary plaques, 10% had coronary stenosis, and 2 patients (3%) had ischemia on the exercise-ECG. In comparison, the prevalence of asymptomatic coronary stenosis investigated with invasive coronary angiography was 47% among 110 pre-transplant patients with type 1 diabetes (170) and 34% in a cohort of 29 asymptomatic diabetics (171). Thus we found a low prevalence of coronary stenosis in our asymptomatic patients with diabetic nephropathy, however pre-transplant patients would be expected to be sicker and methodology differences may as well account for some of the differences between the studies together with the introduction of statins and aspirins in 2002 to all patients with diabetic nephropathy at the Steno Diabetes Center.
In contrast to the large differences in coronary plaque burden, between patients with diabetic nephropathy and persistent normoalbuminuria, aortic plaque burden was similar between groups. Here our data was comparable to a group of asymptomatic subjects from the Framingham Heart Study offspring cohort (172).
The large difference between coronary plaques and aortic plaques is in contrast to previous studies where an association between coronary artery stenosis and atherosclerosis in the thoracic aorta have been shown in patients with coronary heart disease (173;174). This might suggest that presence of diabetic nephropathy is associated with a differential impact on aortic and coronary atherosclerosis and that only coronary atherosclerosis is accelerated in subjects with diabetic nephropathy.
Predicting risk in the individual patient is often done with prediction models, however these are based on data from the general population (175) or from patients with type 2 diabetes (176) and therefore might not be accurate in patients with type 1 diabetes (177). Also risk factors are modified by statins, aspirins, and other cardio- and renoprotective medication making it more difficult to assess risk in the individual. This might explain why the coronary plaque burden did not correlate to blood pressure in patients with diabetic nephropathy but only in patients with persistent normoalbuminuria; however plaque burden did correlate to total cholesterol and diabetes duration.
Our patients with diabetic nephropathy had a high prevalence of coronary plaques (76%) despite significant coronary stenosis was only found in 10% of patients. This is due to outward remodelling with preservation of the coronary artery lumen despite of progression of the coronary plaque (Glagov-type (178)). This type of remodelling is associated with morphological predictors of plaque instability (179), and thus our data suggest that the high cardiovascular mortality and morbidity we found in patients with type 1 diabetes and diabetic nephropathy might be due to coronary heart disease. The low number of coronary stenosis (10%) in patients with diabetic nephropathy and no symptoms or signs of coronary heart disease indicate that non-invasive screening for coronary heart disease might not be suitable for risk assessment. However exercise-ECG could not be used either in this population. The prognostic value of the increased coronary plaque burden awaits follow-up examination of our population.
One limitation to our study could be that our patients had a long duration of diabetes and a relative long duration of diabetic nephropathy. Thus there could be a selection bias in our cohort with long-term survivors represented more than patients dying of early.
This study (6) concludes that asymptomatic type 1 diabetic patients with and without diabetic nephropathy have a low prevalence of coronary stenosis, however coronary plaque burden was increased in patients with diabetic nephropathy and this could very well contribute to the increased prevalence of cardiovascular mortality and morbidity in type 1 diabetic patients.

Left ventricular mass and left ventricular hypertrophy

Hypertension is intimately related to increased LVM and LVH, which causes increased myocardial fibrosis (180), increased risk of myocardial ischemia, coronary heart disease, and heart failure (181). In patients with diabetes, the prevalence of hypertension is increased two to three times compared to non-diabetics, and hypertension is even more frequent in patients with diabetic nephropathy (182;183). Hypertension causing LVH is intimately related to the increased cardiovascular mortality and morbidity seen in patients with diabetes. In the Framingham study, LVH was found to be a strong predictor of cardiovascular disease and death even in asymptomatic patients with normal blood pressure (184). Furthermore LVH is a modifiable risk factor, and regression of LVH with antihypertensive therapy has been shown to improve prognosis, while lack of regression increases cardiovascular risk (185). In the HOPE trial and in the LIFE study it was further more shown that drugs blocking the renin angiotensin system reduces LVM more than other types of drugs, and more than just the effect from lowering blood pressure (186;187). Thus treatment decreasing LVM is possible, and exceeds just lowering of blood pressure.
In our cross sectional study of patients with type 1 diabetes with or without diabetic nephropathy we examined the prevalence of heart disease in asymptomatic patients (6), and since cardiovascular magnetic resonance imaging is the gold standard for evaluation of left ventricular function and anatomy (45) we had the opportunity to evaluate LVM and hypertrophy with a very precise technique (7). Previously, a study from the Steno Diabetes Center showed that patients with nephropathy had a larger LVM and a higher prevalence of LVH than patients with normoalbuminuria (161). This earlier study was made with echocardiography and the average blood pressure in patients with diabetic nephropathy in that study was higher than in our study. This could explain why we found no patients to have LVH in our study according to normal ranges for LVM values (188).
In our study (7), patients with nephropathy had considerably lower systolic blood pressure and diastolic blood pressure compared to earlier studies (161) due to aggressive antihypertensive treatment and would be less likely to have LVH. Therefore, our study reflects cardiovascular function in asymptomatic type 1

diabetics on contemporary reno- and cardio protective medication and our findings emphasize the beneficial effect of intensive blood pressure reduction in diabetic patients (162). However even though hypertrophy might not be present in this population, increased LVM within normal limits is most likely still a predictor of poor outcome. This was supported by the correlation to NT-proBNP, which have been proven to be a strong risk factor in patients with diabetes (189-191).
In our long-term follow-up study in type 2 diabetic patients (8), an echocardiography was made at baseline and 75% of patients with diabetic nephropathy and 51% of patients with persistent normoalbuminuria had LVH. Not surprisingly, this was a significant predictor of all-cause mortality.

RISK FACTORS MEASURED IN BLOOD:

In the INTERHEART study, the effect of potentially modifiable risk factors on risk of myocardial infarction was evaluated in a multicenter observational study including data from 52 countries. The study concluded that well known traditional risk factors (smoking, apolipoproteins, hypertension, diabetes, abdominal obesity, psychosocial factors, diet, alcohol consumption, physical activity) accounted for 90% of risk in men and 94% of risk in women (192). Given these data, one could question the value of biomarkers. However modifiable risk factors do not provide an insight to patophysiological mechanisms, and should be considered either an outcome of patophysiological processes (hypertension, obesity, diabetes), or a factor causing processes that leads to disease (smoking, wrong diet, alcohol consumption). Some mediators of these processes are biomarkers which help us in the understanding of the underlying mechanisms, and thus exhibit potential points of targets for treatment. Seen in this light biomarkers brings understanding of disease, and gives new ideas for treatment regimes. Furthermore recently biomarkers in combination was found to improve risk stratification (193).
We have in several trials evaluated different biomarkers and their prognostic value. Even though these studies are carried out consecutively and thus all biomarkers were not evaluated at one time, the studies provide insight into patophysiological processes, and examine the linkage between the biomarker and hard endpoints. Furthermore in order to conduct trials that intervene towards biomarkers, we need prospective studies showing linkage between biomarkers and specific processes and hard endpoints. One recent study, the JUPITER study, examined the effect of treating men and women with elevated CRP but without dyslipidaemia with rosuvastatin (194). The trial was stopped after nearly 2 years, and showed a reduced incidence of major cardiovascular events in the treated group (194).

Biomarkers of endothelial dysfunction and low-grade inflammation

The pathogenesis of vascular complications involves inflammation and endothelial dysfunction (106-109) and biomarkers of these processes have been shown in the Hoorn Study to explain much of the excess cardiovascular risk in patients with Type 2 diabetes (195;196). In non-diabetic patients and in patients with type 2 diabetes longitudinal studies have demonstrated that inflammatory activity precedes vascular complications (197), and in the same study biomarkers of inflammation was found to have prognostic value in patients with type 2 diabetes. However in type 1 diabetic patients markers of inflammation have only in a cross-sectional study – the EURODIAB Prospective Complications Study – been associated with cardiovascular disease (198). Therefore we conducted a longitudinal study in type 1 diabetic patients to evaluate the prognostic value of biomarkers of endothelial dysfunction and low-grade inflammation (5). In our 10-year prospective follow-up study we tested the hypothesis that markers of inflammation and endothelial dysfunction are associated with all-cause mortality and cardiovascular morbidity and mortality, and furthermore if biomarkers related to progression of diabetic nephropathy (5). The study had three main results. First, in patients with diabetic nephropathy, low-grade inflammation (estimated from a combined Z-score of four markers: C-reactive protein, interleukin-6, soluble intercellular adhesion molecule (sICAM-1), and secreted phospholipase A(2)) was associated with all-cause mortality and the combined cardiovascular endpoint after adjusting for cardiovascular risk factors. However the area under the receiver operating characteristic curve for all-cause mortality for all traditional risk factors used in the Cox model in all patients did not change after adding the Z-scores on top of traditional risk factors. Second, in unadjusted analyses, endothelial dysfunction (estimated from a Z-score of three markers: soluble vascular cell adhesion molecule 1, plasminogen activator inhibitor-1, and sICAM-1; since sICAM-1 is both a marker of inflammation and endothelial dysfunction) was also associated with these endpoints. Third, endothelial dysfunction correlated significantly with the rate of decline of GFR, a measure of progression of diabetic nephropathy.
Inflammation and endothelial dysfunction are thought to be key processes in atherothrombosis (199). Indeed, in type 2 diabetes biomarkers have already been shown in a longitudinal study to progress over time, and to precede vascular complications (197). Our data (5) on inflammation are in line with these concepts. The results for endothelial dysfunction were less clear but should not be interpreted to indicate that endothelial dysfunction is not involved in the development of cardiovascular disease. In fact, endothelial dysfunction was strongly correlated with cardiovascular risk factors at baseline, in agreement with previous data (198), and to mortality outcome in unadjusted analyses. Because cardiovascular risk factors cause endothelial dysfunction a Cox regression model adjusting for all traditional cardiovascular risk factors might be overadjusted, and this might explain why endothelial dysfunction did not come out as a significant predictor in the Cox regression analysis. Thus a reasonable interpretation is that our results are compatible with a role for endothelial dysfunction, as has been suggested before (200;201). Additional reasons why the association between endothelial dysfunction and mortality may have been underestimated include the fact that we included only three biomarkers (data on more specific endothelial biomarkers such as sE-selectin and von Willebrand factor not being available) and that we were unable to include a more direct estimate of endothelial nitric oxide availability, e.g. by measuring flow-mediated endothelium-dependent vasodilatation (200;202).
The study showed endothelial dysfunction to correlate with progression of renal disease throughout the follow-up period indicating that endothelial dysfunction is an active part of the pathophysiology causing further progression of the glomerulosclerosis and not just in initiating disease (5). This is an important and novel finding in patients with type 1 diabetes. Endothelial dysfunction has previously in patients with type 2 diabetes been shown to predict progression of renal disease (203). Intervention studies towards endothelial dysfunction in regards to decline in kidney function must be the next step.

With the new knowledge of the association of biomarkers with hard endpoints in type 1 diabetes added by our study (5), together with prognostic data in type 2 diabetic patients (195;197), and a recent post hoc analysis of the IRMA-2 trial showing irbesartan reduces inflammatory activity in patients with type 2 diabetes and microalbuminuria (204) the next step is trials with intention to alter inflammation and endothelial dysfunction as suggested in a statement by the American Heart Association (205). Recently the JUPITER trial addressed this and found benefit when treating individuals with elevated CRP (194). Patients with type 1 diabetes and nephropathy provide a group of patients in whom intervention towards inflammation and endothelial dysfunction could be targeted since we show Z-scores of inflammation and endothelial dysfunction to be associated with strong baseline risk factors, and even though the biomarkers did not add prognostic value on top of known cardiovascular risk factors they do provide insights to the pathophysiology of cardiovascular disease.

NT-proBNP

In the Heart Outcomes Prevention Evaluation (HOPE) study the impact of multiple biomarkers including NT-proBNP on top of traditional risk factors was evaluated in patients known to have cardiovascular disease. The original hypothesis of this sub-study was that a multimarker strategy including different patophysiological processes would come out as the most informative clinical tool, however the study did not support this, and the only biomarker that was shown to add prognostic value to traditional risk factors was NT-proBNP (206).
Brain natriuretic peptide (BNP) is synthesized as a prehormone, and then cleaved into N-terminal ProBNP (NT-proBNP) and BNP. NT-proBNP is the more stable compound and BNP is released in response to left ventricular myocyte stretch (207). NT-proBNP and BNP seems to provide equally information (208). Natriuretic peptides has been shown to have strong prognostic value in non-diabetic populations (209), and in patients with hypertensive kidney disease (210). Also in patients with diabetes the prognostic value is well established. NT-proBNP has been shown to predict cardiovascular mortality and morbidity in type 2 diabetic patients with microalbuminuria (191), and to predict mortality in patients with type 2 diabetes and macroalbuminuria, microalbuminuria, and normoalbuminuria (190), and in type 1 diabetes NT-proBNP has also been shown to predict cardiovascular mortality and morbidity in patients with diabetic nephropathy (189). In type 2 diabetic patients with normoalbuminuria NT-proBNP was increased in patients with hypertension (211) and NT-proBNP was related to LVH. In the Losartan Intervention For Endpoint Reduction Hypertension (LIFE) study NT-proBNP predicted cardiovascular events in patients with LVH and hypertension (212). However, in most studies the association between LVM and NT-proBNP is based on measurements of LVM done with echocardiography and relations between LVM and NT-proBNP is described in patients with hypertrophy and/or hypertension. We sought to evaluate CMR measurements, very accurate measures of left ventricular function and mass, and their correlation to NT-proBNP in type 1 diabetic patients with and without diabetic nephropathy and without evidence or history of cardiac disease (7).
None of the patients had LVH, actually the LVM was matching non-diabetic populations (45;188). Levels of NT-proBNP were significantly increased in patients with nephropathy compared to normoalbuminuric patients (7). Furthermore, NT-proBNP was independently predictive of increased LVM in patients with diabetic nephropathy, together with creatinine, but not in patients with normoalbuminuria (7). None of the CMR measures of volumes correlated to levels of NT-proBNP in patients with nephropathy or in patients with normoalbuminuria. The finding that NT-proBNP in patients without known cardiovascular disease and without LVH still is related to LVM in patients with diabetic nephropathy is probably an indicator of the overall high cardiovascular risk seen in these patients (1).

PAPP-A and PlGF

PAPP-A:
Pregnancy-Associated Plasma Protein A (PAPP-A) belongs to the matrix metalloproteinase family known to degrade extra-cellular matrix and thereby contribute to the fragility of the lipid rich plaque leading to rupture. Matrix metalloproteinases are overexpressed in atherosclerotic lesions and makes these lesions fragile leading to rupture. PAPP-A is typically measured during pregnancy in maternal blood to screen for Down syndrome, but PAPP-A is present physiologically in both men and women and constitutes an activator of insulin-like growth factor, a mediator of atherosclerosis (213). PAPP-A is also expressed in atherosclerotic lesions (213), and circulating PAPP-A levels have been proposed to be a biological marker of artherosclerosis and unstable plaques causing acute coronary syndromes (214;215). Levels of PAPP-A is also shown to be correlated with plaque complexity in stable patients (215) and recently PAPP-A was shown to predict all-cause mortality in patients with chronic stable angina pectoris (216). Patients with diabetic nephropathy are known to have a high cardiovascular risk (1;11) and we therefore evaluated PAPP-A as a predictive marker of all-cause mortality and cardiovascular mortality and morbidity in our cohort of 401 type 1 diabetic patients including 200 patients with diabetic nephropathy followed for approximately 10 years. However we could only measure PAPP-A in 375 patients, including 197 patients with diabetic nephropathy (4).
We found circulating levels of PAPP-A, above the suggested threshold value of 10 mIU/L for unstable coronary plaques (214), to predict all-cause mortality after adjustment for presence of nephropathy (4). However results were no longer significant after adjusting for traditional cardiovascular risk factors. In patients with overt diabetic nephropathy levels of PAPP-A was increased compared to normoalbuminuric patients and the prognostic value of PAPP-A was much stronger in patients with nephropathy, however in multivariate analysis PAPP-A was not an independent predictor of all-cause mortality or cardiovascular mortality and morbidity. In patients with diabetic nephropathy the independent predictive value of PAPP-A was borderline significant in regards to all-cause mortality.
In non-diabetic populations circulating levels of PAPP-A are elevated in acute coronary syndromes (214;217) and also predict outcome in these patients (218;219). Our study indicates that PAPP-A also reflects overall risk in patients not acutely ill from cardiovascular disease. Earlier cross sectional studies of PAPP-A have also found increased PAPP-A levels in patients with an increased cardiovascular risk, i.e. carotid intima media thickness (IMT) a surrogate endpoint for cardiovascular risk (220) was found to be associated with PAPP-A in type 2 diabetic patients (221). Furthermore PAPP-A was elevated in hypercholesterolemic subjects with type 2 diabetes (221) and in hypercholesterolemic subjects without diabetes (222). Prospective studies of PAPP-A have until now only been performed in patients diagnosed with acute coronary syndromes (218;219). Our results and the association observed in cross sectional studies between PAPP-A and IMT (221) and hypercholesterolemia (222) indicate that PAPP-A not

only is increased in acute cardiovascular syndromes but increased PAPP-A levels also serve as a marker of the total atherosclerotic burden and cardiovascular risk. In agreement with this hypothesis PAPP-A levels in pre-transplant kidney patients was predictive of post-transplant cardiovascular disease (223) in a non diabetic population perhaps indicating a state of inflammation in patients with increased PAPP-A values.
In conclusion the study of PAPP-A as a risk marker showed that our high risk patients with diabetic nephropathy had a higher level of PAPP-A than the normoalbuminuric group, and PAPP-A was in a model adjusted only for patients with nephropathy predictive of all-cause mortality - reflecting the increased cardiovascular risk in patients with diabetic nephropathy.

PlGF:
Placental growth factor (PlGF) was originally identified in the placenta (224) and PlGF stimulates vascular smooth cell growth and is a member of the vascular endothelial growth factor (VEGF) family. As reviewed by Autiero al (225), PlGF is found primarily in the placenta, however PlGF is also expressed in the heart, lung, thyroid gland, and skeletal muscle and interestingly it seems that PlGF and its receptor VEGFR-1 is only active in angiogenesis under pathological conditions. PlGF seems to play a functional role in the patophysiological angiogenesis occurring in cancer diseases, rheumatoid arthritis, and ischemic retinopathy, however this has only been shown in animal studies where blocking of the VEGFR-1 was effective in attenuating pathological vessel formation (226). PlGF has also been shown to be relieving limb ischemia by formation of new vessels (226). A blockade of the angiogenesis driven by PlGF and its receptor could potentially be of benefit in many cancer diseases, and most blockers have been developed and tested for use in cancer trials. However, it has been proven that blocking of VEGFR-1 reduced the atherosclerotic plaque growth and vulnerability (226), thus it is possible that PlGF play a role in the development of cardiovascular disease through inflammatory pathways and thus stimulation of atherosclerotic plaque formations. Since PlGF is accessible for intervention it is highly relevant to examine the prognostic value of PlGF in patients known to be at high risk of cardiovascular disease. Therefore we examined the prognostic value of PlGF in our cohort of type 1 diabetic patients with and without diabetic nephropathy (2). A combined endpoint of cardiovascular mortality and morbidity was the primary endpoint. The analysis was restricted to patients with diabetic nephropathy.
PlGF was found to significantly predict the cardiovascular mortality and morbidity in patients with diabetic nephropathy even after adjusting for strong traditional risk factors and kidney function (2). From animal studies PlGF seems to play a role in vascular inflammation (226), and our results with PlGF predicting cardiovascular mortality and morbidity support these studies. Patients with diabetes and diabetic nephropathy could be a potential group of patients to target with blocking of PlGF in order to reduce cardiovascular mortality and morbidity especially with our data documenting prognostic value of PlGF.

Transforming growth factor-Beta

TGF-β is a cytokine involved in fibrosis, and thus a number of human diseases including diabetic nephropathy (227). From animal models TGF-β is believed to act as a mediator of renal fibrosis (228). TGF-β stimulates matrix production and seems to block degradation of matrix resulting in increased fibrogenesis (229). In diabetic mice, TGF- β antibodies have proven to prevent mesangial matrix expansion and thus attenuate the pathogenesis of glomerulosclerosis (230). Also in man TGF-β antibodies seems to be effective as antifibrotic therapy in renal diseases at least when combined with RAS blockade (231). As suggested recently by Remuzzi and Perico, anti-TGF-β could have a potential role in the management of patients with diabetic nephropathy as a part of a multifactorial treatment regime (232). When choosing patient groups to target with this new intervention it seems reasonable to identify groups in which TGF-β is shown to either enhance the progression of disease or high levels of TGF-β is correlated to hard endpoints. Thus we examined the influence of TGF-β in a 10-year prospective follow-up study of type 1 diabetic patients with and without diabetic nephropathy (5). We tested the hypothesis that TGF-β was associated with all-cause mortality and cardiovascular mortality and morbidity and/or was related to progression of diabetic nephropathy (5). Our results on TGF-β were negative in all regards. However, we cannot exclude a local effect in the kidney of TGF-β. TGF-β levels were significantly higher at baseline in patients with diabetic nephropathy and perhaps most of the role of TGF-β is played before the clinical diagnosis of diabetic nephropathy is evident. Our negative results on TGF-β should be taken into consideration when patients are chosen for trials with intervention towards TGF-β and thus patients with less progressed disease might provide the correct target group. It might be that intervention with anti-TGF-β could provide, together with blockade of the RAS, further protection from progression to overt diabetic nephropathy together with multifactorial treatment with statins, aspirin, smoking cessation, and lifestyle change.

Lipids, triglycerides, and hyperglycaemia.

As described under the section “multifactorial aggressive treatment and lifetime risk” dyslipidaemia and hyperglycaemia represents strong cardiovascular risk factors.
In the follow-up study in type 1 diabetes describing the improved prognosis in type diabetic patients with diabetic nephropathy (1), glycaemic control (HbA1c) was found to be a predictor of the combined endpoint of cardiovascular morbidity and mortality, however only if patients with a history of stroke or myocardial infarction at baseline was excluded from the analysis. Interestingly survivors through follow-up had a marked decrease in HbA1c and a better lipid profile at the follow-up examination than on baseline, and urinary albumin excretion was reduced together with blood pressure reflecting the enhanced treatment of risk factors in patients with diabetic nephropathy during the 10 years of the follow-up period. Neither total cholesterol nor any of the individual lipids was predictive of the combined cardiovascular endpoint, however total cholesterol and HbA1c was significant predictors of all-cause mortality (4) in this population.
In type 2 diabetic patients we found HbA1c to be a significant predictor of all-cause mortality together with other well documented risk factors, however lipids did not come out as a independent risk factor (8).
A high level of triglycerides is part of the atherogenic dyslipidaemia in the metabolic syndrome and in type 2 diabetes. In large studies the predicting value of high triglycerides has been linked to the profile of other measures of dyslipidaemia such as the LDL/HDL ratio. This was found in both the Prospective Cardiovascular Munster (PROCAM) (233) and in the Helsinki Heart Study (234). Thus triglycerides is be appropriate to evaluate as part of a dyslipidaemic profile, however the expected effect of treatment of dyslipidaemia might be highest with the higher levels of triglycerides as observed in the Helsinki Heart Study (234). In this thesis

triglycerides have not been evaluated per se as a risk factor since we only chose one factor representing dyslipidaemia in the analysis. And since total cholesterol was the stronger factor in our follow-up studies triglyceride did not enter the models.

Non-modifiable risk factors:
Genetic risk factors

Family clustering of cardiovascular disease support the hypothesis that genetics are initiating and or enhancing processes that eventually leads to cardiovascular disease. Even though traditional risk factors was estimated to account for up till approximately 90% of risk of myocardial infarction in the INTERHEART study, many of these traditional risk factors are to a certain extend inherited (diabetes, obesity, psychosocial factors). However genetic risk factors of cardiovascular disease have not been the subject of the studies included in this thesis, and furthermore have been reviewed recently (235;236).

CONCLUSIONS AND SUMMARY

The prevalence of diabetes mellitus is increasing worldwide. The consequences are considerable in regards to loss of working power and increasing expenses in health care, and thus diabetes is a major economical burden for the society. On an individual basis, diabetes causes shortening of expected life length and poorer life quality. The treatment of diabetes has changed radically over the last century, with patients today struggling with complications to diabetes, and to a smaller extends the acute illness. The most devastating microvascular complication is still diabetic nephropathy, and without specific intervention 20-40 percent of all patients develop this complication. The development of diabetic nephropathy is characterized by hypertension, increasing albuminuria, and a progressive decline in GFR. Today, patients have a longer life expectancy and an increasing risk of developing and dying from cardiovascular disease. Accordingly, the focus of treatment today has been adjusted to target not only diabetic nephropathy but also treatment is targeted towards the increasing cardiovascular risk in these patients. As a consequence the treatment of patients with diabetes today has become multifactorial.
This thesis focuses on prognosis, prevalence of cardiovascular disease, and identification of cardiovascular risk factors.
To assess prognosis a long-term observational case-control study in type 1 diabetic patients with or without diabetic nephropathy was carried out. Compared to earlier studies the prognosis was found to have improved. This is an important finding, since the efforts of treatment has changed in the same decade with even more aggressive antihypertensive treatment, and the introduction of statins, and aspirin. However we underline that prognosis most likely will improve even further since treatment of risk factors have changed in our patient cohort during follow-up. In the same study 40% of patients developed or died from cardiovascular disease during follow-up compared to 10% with persistent normoalbuminuria. Therefore the next step was to examine the prevalence of cardiovascular disease in asymptomatic patients, since cardiovascular disease often is silent in patients with diabetes and the lesions advanced at time for diagnosis. Therefore a cross-sectional study including type 1 diabetic patients with or without diabetic nephropathy without signs or symptoms of cardiovascular disease was carried out. In patients with diabetic nephropathy a high prevalence of coronary plaques was found, however only a few patients had significant coronary stenosis. Since presence of plaques is closely related to clinical disease, the results suggest that part of the increased cardiovascular mortality and morbidity in patients with nephropathy can be explained by these coronary plaques. This case control study also revealed that patients with diabetic nephropathy who were without signs or history of cardiovascular disease did not have LVH. This is in contrast to earlier findings of a high prevalence of LVH in patients with diabetic nephropathy. Most likely our results reflects the aggressive antihypertensive treatment these patients receive, and the corresponding average blood pressure values, which was much lower than in previous cohorts.
In the long-term follow-up study in type 1 diabetic patients cardiovascular risk factors and risk markers was evaluated. PAPP-A and P1GF was shown to have prognostic value in patients with diabetic nephropathy. Furthermore, a combination of inflammatory markers and of markers of endothelial dysfunction was found to be associated with hard endpoints. However, the major prognostic factor was traditional risk factors, since these risk factors could explain up till 90 percent of the overall risk. The study showed markers of endothelial dysfunction to correlate with the rate of decline in GFR, a measure of progression of diabetic nephropathy. In the cross-sectional CMR study the cardiovascular marker NT-proBNP was elevated in patients with diabetic nephropathy and related to LVM, probably this indicates the increased cardiovascular risk in these patients. In the long-term follow-up study in type 2 diabetic patients, LVH was shown to be a significant predictor of all-cause mortality. Today blood pressure is treated to a much lower level and thus LVH is less common in patients receiving the optimal treatment.
In type 2 diabetic patients non-dipping of night blood pressure has been ascribed in part by vagal dysfunction and increased sympathetic nervous function, caused by autonomic neuropathy, but the consequence of the non-dipping phenomenon was unknown. The follow-up study in type 2 diabetic patients study showed that non-dipping was a risk factor for all-cause mortality. In the same follow-up study decreasing heart rate variation indicating cardiac autonomic neuropathy was also a significant predictor of all-cause mortality. In type 1 diabetic patients we have also shown decreased heart rate variation to be a significant predictor of the cardiovascular mortality and morbidity.
The improved prognosis in patients developing diabetic nephropathy has revealed the need to target cardiovascular complications to improve life length and quality of life even further. Because of medical intervention, cardiovascular risk in diabetic patients with nephropathy is becoming more difficult to evaluate from traditional risk factors, such as cholesterol, LVH, and blood pressures and it might be that new risk factors must be used in order to find high risk individuals. The multifactorial treatment regimes can still be improved and new targets can be found in order to prevent and postpone both diabetic and cardiovascular complications. The studies in this thesis have added in terms of showing the improved prognosis with more aggressive treatment, and evaluating new and traditional risk factors and biomarkers, and early surrogate measures such as plaque burden for increased risk in order to identify high risk individuals early.

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