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Markers of inflammation and endothelial dysfunction are associated with incident cardiovascular disease, all-cause mortality, and progression of coronary calcification in type 2 diabetic patients with microalbuminuria

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ABSTRACT

Background: We evaluated markers of inflammation and endothelial dysfunction and their associations with incident cardiovascular disease (CVD), all-cause mortality and progression of coronary artery calcium (CAC) in patients with type 2 diabetes (T2D) and microalbuminuria but without known coronary artery disease (CAD).

Methods: Prospective study including 200 patients receiving multifactorial treatment. Markers of inflammation (TNF-α, sICAM-1, sICAM-3, hsCRP, SAA, IL-1β, IL-6, IL-8) and endothelial dysfunction (thrombomodulin, sVCAM-1, sICAM-1, sICAM-3, sE-selectin, sP-selectin) were measured at baseline. Adjustment included traditional CVD risk factors, and full adjustment additionally NT-proBNP and CAC. The "SQRT method" assessed CAC progression after 5.8 years, and cut-point was an annualised difference >2.5.

Results: Occurrence of CVD ($n = 40$) and all-cause mortality ($n = 26$) was traced after 6.1 years. In adjusted and fully adjusted Cox models, TNF-α was a determinant of CVD and all-cause mortality ($p \le 0.007$). Further, in adjusted and fully adjusted logistic regression, TNF-α was related to CAC progression ($p \le 0.042$). Of the other biomarkers, sICAM-3 and thrombomodulin were also associated with both endpoints ($p \le 0.046$), IL-1β with CVD endpoints ($p = 0.021$), and sVCAM-1 and sICAM-1 with all-cause mortality ($p \le 0.005$). Higher composite z-scores including all markers of inflammation and endothelial dysfunction were associated with CVD and all-cause mortality ($p \le 0.008$).

Conclusions: In patients with T2D and microalbuminuria without known CAD and receiving multifactorial treatment, biomarkers of inflammation and endothelial dysfunction were independently associated with CVD, all-cause mortality and CAC progression. Especially TNF-α was a robust determinant, even after adjusting for NT-proBNP and CAC.

1. Introduction

Individuals with type 2 diabetes, and especially those with concomitant albuminuria, are at high risk of cardiovascular disease (CVD) (Garg & Bakris, 2002; Haffner, Lehto, Ronnemaa, Pyorala, & Laakso, 1998). Endothelial dysfunction is considered one of the earliest markers of atherosclerosis, and it is well documented that inflammatory processes play an important role in the causation of atherosclerotic CVD (Libby, 2002). Inflammatory mediators play a paramount role in the initiation, progression and rupture of atherosclerotic plaques. Thus, markers of inflammation and endothelial dysfunction may provide additional information about the risk of developing CVD and may become new targets for treatment. In previous studies, we have shown markers of endothelial dysfunction and inflammation to be strongly and independently associated with

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CVD and mortality (de Jager, Dekker, Kooy, et al., 2006; Stehouwer et al., 2002). Intensified, targeted, multifactorial intervention in type 2 diabetes has subsequently been shown to reduce risk of CVD compared to conventional intervention (Gaede et al., 2003). However, it is not known whether such treatment affects endothelial dysfunction and inflammation. If multifactorial treatment does have an effect, then markers of endothelial dysfunction and inflammation would be expected to be less associated with CVD and all-cause mortality in these patients.

The per-protocol pre-specified aim of this prospective study was to evaluate established and novel markers of low-grade inflammation and endothelial dysfunction as determinants of combined fatal and non-fatal CVD and all-cause mortality in patients with type 2 diabetes and microalbuminuria, but without known CAD and receiving multifactorial treatment. Moreover, as tertiary endpoint it was also pre-specified to evaluate the relation between markers of endothelial dysfunction and inflammation and the progression in coronary artery calcium score (CAC) in patients without fatalities.

2. Methods

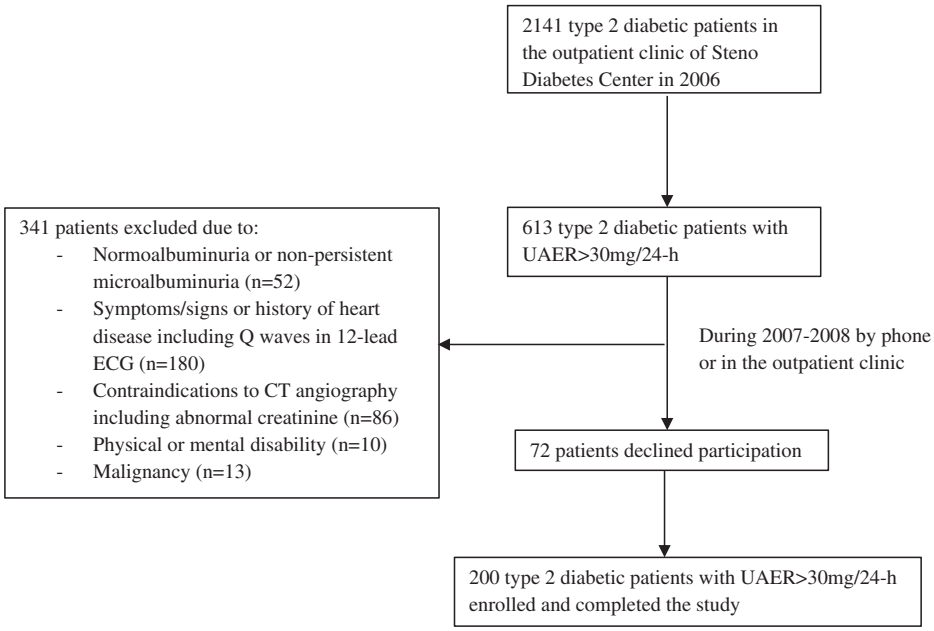
2.1. Participants and study procedure

At Steno Diabetes Center, we identified, from January 2007 to February 2008, a consecutive cohort of 200 outpatients with type 2 diabetes treated in a secondary care setting. All patients received treatment with multifactorial intervention constituting of glycemic, lipid and blood pressure control, as well as antithrombotic therapy and lifestyle modification according to the Steno-2 study (Gaede et al., 2003). Patients were included if they met the following inclusion criteria: 1) outpatients with type 2 diabetes defined according to WHO criteria; 2) no history of CAD or other cardiac disease and without any symptoms from the heart, assessed from patient files and thorough interviews and questionnaires; 3) persistent (two out of three consecutive measurements) urinary albumin excretion rate (UAER) >30 mg/24 h.

A written invitation was sent to 613 consecutive patients (69% males and a mean (standard deviation [SD]) age of 47 (8) years). A total of 72 patients refused to participate. Furthermore, patients ($n = 341$) were excluded (either by phone interview or after examination in the outpatient clinic) if one or more of the following characteristics were present: 1) normal UAER or non-persistently elevated UAER ($n = 52$); 2) symptoms/signs or history of heart disease including Q waves in 12-lead ECG ($n = 180$); 3) relative contraindications to CT angiography (CTA) or CAG, including abnormal plasma creatinine levels ($n = 86$); 4) physical or mental disability ($n = 10$); or 5) malignancy ($n = 13$). Thus, the final study population included 200 patients. A detailed flow chart of the selection of the study population is shown in Fig. 1. The a priori sample size calculation was based on the assumption that 20%–30% of the patients would experience a cardiovascular event during 5 years of follow-up (Adler et al., 2003). This study complies with the Declaration of Helsinki, the research protocol was approved by the local ethics committee and all patients gave written informed consent.

2.2. Biochemical analyses and other

Measuring of the following biomarkers was pre-specified: tumor necrosis factor alpha (TNF-α), soluble intercellular adhesion molecule 1 (sICAM-1), soluble intercellular adhesion molecule 3 (sICAM-3), high-sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), soluble thrombomodulin, soluble vascular cell adhesion protein 1 (sVCAM-1), soluble E-selectin and soluble P-selectin. MSD multipanel measurements or enzyme-linked immunosorbent assay (ELISA) were applied. NT-proBNP was measured in all patients and analyzed by an immunoassay as previously described (Tarnow, Gall, Hansen, Hovind, & Parving, 2006). UAER was measured in 24-h urine collections by an enzyme immunoassay (Reinhard, Hansen, Persson, et al., 2011). Current smoking was defined as one or more cigarettes/cigars/pipes a day.



Selection of the final study population.

Fig. 1. Flow chart. Selection of the final study population.

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