Acta Diabetologica (2019) 56:711–712
https://doi.org/10.1007/s00592-019-01306-9

SHORT COMMUNICATION

A marker of type VI collagen formation (PRO-C6) is associated with higher arterial stiffness in type 1 diabetes

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Received: 12 December 2018 / Accepted: 13 February 2019 / Published online: 9 March 2019

Persons with type 1 diabetes (T1D) have increased risk of cardiovascular disease. Large artery stiffness is an important determinant of cardiovascular risk. Carotid–femoral pulse wave velocity (cfPWV) is considered the gold standard measure of arterial stiffness, and has been shown to be a strong predictor of mortality and cardiovascular outcome [1].

Arterial stiffening reflects fragmentation and loss of elastin fibers and accumulation of collagen fibers in the media of large arteries. However, the mechanisms responsible for arterial stiffening remain incompletely understood [2].

In this cross-sectional study, we measured two biomarkers reflecting collagen formation and degradation, namely PRO-C6, which detects the C-terminal pro-peptide of type VI collagen released upon normal maturation of the collagen and C3M which is a specific fragment of type III collagen generated by matrix metalloproteinase 9 [3, 4]. High serological levels of PRO-C6 and C3M have been observed in patients with cardiovascular disease [4]. However, the association between cfPWV and PRO-C6 and C3M has never been evaluated before. We, therefore, evaluated the associations between cfPWV and PRO-C6 and C3M measured in serum and urine in 634 persons with T1D and various degrees of diabetic nephropathy. The participants were stratified according to different stages of nephropathy as one of the aims of the overall study was to examine the relation between arterial stiffness and diabetes complications [5].

The participants were recruited from the outpatient clinic at Steno Diabetes Center Copenhagen, Denmark. Written informed patient consent and ethical approval of the study were obtained. The details of the study have previously been described [5]. PRO-C6 and C3M in serum and urine were measured with the enzyme-linked immunosorbent assay (ELISA) method developed by Nordic Bioscience, Denmark [3, 4]. cfPWV was measured with the SphygmoCor device (Actor Medical, Sydney, Australia) by trained laboratory technicians following 15 min of supine rest. Three measurements were performed and averaged.

We applied unadjusted and adjusted linear regression analyses. Adjustment included sex, age, mean arterial pressure, LDL cholesterol, smoking, HbA1c, treatment with renin–angiotensin–aldosterone system blockers, estimated glomerular filtration rate (eGFR) and urinary albumin excretion rate (UACR). To adjust for urine output levels, the urinary markers were normalized for urinary creatinine.

We tested for effect modulation in the associations for the influence of different stages of nephropathy by introducing the appropriate interaction term in the regression models. Of the 634 participants, 349 (55%) were male, mean ± SD age was 54.6 ± 12.6 years, eGFR 81 ± 26 ml/min/1.73 m^2, systolic blood pressure 132 ± 17 mmHg and cfPWV 10.4 ± 3.3 m/s.

Table 1 shows the unadjusted and adjusted associations between cfPWV and the biomarkers.

Higher serum and urinary levels of the collagen formation marker PRO-C6 were associated with higher cfPWV in unadjusted models ($p \leq 0.039$). After adjustment only higher serum level remained significantly associated with higher cfPWV (β estimate per doubling of serum PRO-C6: 0.48 ± 0.21; $p = 0.026$).

Higher serum level of the collagen degradation marker C3M was associated with higher cfPWV in the unadjusted model ($p = 0.002$), but significance was lost after adjustment ($p = 0.24$). Higher urinary level of C3M was associated with lower cfPWV in the unadjusted model ($p < 0.001$), but significance was lost after adjustment ($p = 0.44$).

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Table 1 Associations between the biomarkers and carotid–femoral pulse wave velocity (cfPWV)

Biomarker (median [IQR])	Model	cfPWV	*p*
Serum PRO-C6 (7.5 [5.9–10.1]) ng/ml	Unadjusted	1.54 ± 0.19	**< 0.001**
	Adjusted	0.48 ± 0.21	**0.026**
Serum C3M (87.5 [7.1–10.3]) ng/ml	Unadjusted	0.67 ± 0.29	**0.002**
	Adjusted	0.26 ± 0.22	0.24
Urinary PRO-C6 (0.21 [0.17–0.27]) ng/µmol	Unadjusted	0.34 ± 0.16	**0.039**
	Adjusted	−0.16 ± 0.13	0.31
Urinary C3M (5.57 [3.74–7.48]) ng/µmol	Unadjusted	−1.03 ± 0.18	**< 0.001**
	Adjusted	−0.10 ± 0.16	0.44

The β estimates represent a doubling of the biomarkers. Adjustment included sex, age, mean arterial pressure, LDL cholesterol, smoking, HbA1c, treatment with renin–angiotensin–aldosterone system blockers, eGFR and urinary albumin excretion rate. Numbers in bold indicate *p*-values < 0.05

None of the tests for effect modulation in relation to the influence of different stages of nephropathy reached significance, neither in unadjusted nor adjusted analyses ($0.11 < p < 0.44$).

In the search for a link between collagen formation and vascular disease, we demonstrated that higher level of a fragment reflecting type VI collagen formation, namely serum PRO-C6, was independently associated with arterial stiffness in persons with T1D. In contrast, the association between urinary level of PRO-C6 and both serological and urinary levels of C3M (representing type III collagen degradation) and arterial stiffness was lost after adjustment for traditional risk factors.

The study consisted of a large and well-characterized group of persons with T1D. Arterial stiffness was evaluated with the gold standard method, analyzed as a continuous variable and with proper adjustment.

In conclusion, in type 1 diabetes, higher serum level of PRO-C6, a marker of collagen type VI formation, was associated with increased arterial stiffness. This observation could introduce a new marker to monitor the therapeutic efficacy of antifibrotic agents such as tranilast and its analogues and potentially reduce the cardiovascular risk in type 1 diabetic persons with increased arterial stiffness.

Author contributions MF, TWH, PR, DGKR and FG conceived and designed the letter. MF and TWH drafted the manuscript. PR, DGKR and FG critically revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest DGKR, SHN, MAK, and FG are full-time employees of Nordic Bioscience. Nordic Bioscience is a privately owned, small- to medium-sized enterprise partly focused on the development of biomarkers. None of the authors received fees, bonuses, or other benefits for the work described in the article. MAK and FG hold stocks in Nordic Bioscience. The patent for the ELISAs used to measure PRO-C6 and C3M levels is owned by Nordic Bioscience. The funder provided support in the form of salaries for DGKR, SHN, MAK, and FG but did not play any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. There are no conflicts of interest to disclose for PR, ST, TWH or MF.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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