



Carotid intima-media thickness is a predictor of subclinical myocardial damage in men with type 2 diabetes mellitus

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Keywords:	Diabetes < Autoimmunity, Cardiovascular risk management < Cardiovascular incidences, Coronary artery disease < Cardiovascular incidences
Abstract:	<p>Background: Type 2 diabetes mellitus (T2DM) promotes the development of atherosclerosis and is a major risk factor for cardiovascular disease. High-sensitivity cardiac troponin I (hs-cTnI) assays fundamentally improved the diagnosis of myocardial injury and even enable the prediction of future cardiovascular events in the general population. However, data about the association of hs-cTnI with cardiovascular risk factors and carotid intima media thickness (cIMT) as a marker of atherosclerosis are limited, especially in patients with T2DM.</p> <p>Methods: In this cross-sectional study we analyzed clinical and laboratory parameters of 234 patients (43% women) with T2DM and a median age of 65 years (interquartile range: 57-71). The median duration of diabetes mellitus was 10 years (6-17). Anthropometric data, blood pressure, glycemic parameters and lipid profiles were determined. Hs-cTnI plasma concentrations were measured on an ADVIA Centaur XPT immunoassay analyzer and cIMT was evaluated by high-resolution ultrasound.</p> <p>Results: Hs-cTnI plasma concentrations were below the gender-specific 99th percentiles in 93% of T2DM patients with a median concentration of 4.0 ng/l (interquartile range: 2.0-10.0). Hs-cTnI was significantly associated with gender, renal function and C-reactive protein in the entire study cohort. Gender-specific analyses revealed cIMT and renal</p>

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	function to be significantly associated with hs-cTnI in men. Contrary, only age was significantly associated with hs-cTnI in women. Conclusion: In a real-world clinical setting in patients with T2DM, cIMT is a predictor of subclinical myocardial damage in men, but not in women.



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9 **Running title:** High-sensitivity cardiac troponin I in type 2 diabetes mellitus
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Abstract

Background: Type 2 diabetes mellitus (T2DM) promotes the development of atherosclerosis and is a major risk factor for cardiovascular disease. High-sensitivity cardiac troponin I (hs-cTnI) assays fundamentally improved the diagnosis of myocardial injury and even enable the prediction of future cardiovascular events in the general population. However, data about the association of hs-cTnI with cardiovascular risk factors and carotid intima media thickness (cIMT) as a marker of atherosclerosis are limited, especially in patients with T2DM.

Methods: In this cross-sectional study we analyzed clinical and laboratory parameters of 234 patients (43% women) with T2DM and a median age of 65 years (interquartile range: 57-71). The median duration of diabetes mellitus was 10 years (6-17). Anthropometric data, blood pressure, glycemic parameters and lipid profiles were determined. Hs-cTnI plasma concentrations were measured on an ADVIA Centaur XPT immunoassay analyzer and cIMT was evaluated by high-resolution ultrasound.

Results: Hs-cTnI plasma concentrations were below the gender-specific 99th percentiles in 93% of T2DM patients with a median concentration of 4.0 ng/l (interquartile range: 2.0-10.0). Hs-cTnI was significantly associated with gender, renal function and C-reactive protein in the entire study cohort. Gender-specific analyses revealed cIMT and renal function to be significantly associated with hs-cTnI in men. Contrary, only age was significantly associated with hs-cTnI in women.

Conclusion: In a real-world clinical setting in patients with T2DM, cIMT is a predictor of subclinical myocardial damage in men, but not in women.

Key words: High-sensitivity cardiac troponin I; type 2 diabetes mellitus; intima-media thickness, cardiovascular disease

Introduction

Type 2 diabetes mellitus (T2DM) promotes the development of atherosclerosis thereby increasing the risk for cardiovascular disease (CVD), the leading cause of morbidity and mortality in T2DM [1,2]. Compared with persons without diabetes, patients with T2DM have a twofold higher risk developing CVD and diabetes-related macrovascular complications. This atherogenic risk is known to be markedly larger in men compared to women [3,4]. Nowadays, the combination of clinical markers with biomarkers allows the identification of individuals at increased CVD. This can enable appropriate treatment tailored to patients with an already existing macrovascular disease, such as coronary heart disease. Cardiac-specific troponins (cTn) are powerful biomarkers to detect subclinical myocardial damage and revolutionized treatment in patients with acute coronary syndrome (ACS) [5-7]. Subclinical myocardial damage leads to release of cardiac troponin I (cTnI) and T (cTnT) that are detectable from the bloodstream. The development and introduction of high-sensitivity assays for the determination of cTn not only fundamentally improved the diagnostic performance and clinical utility, but also allowed the quantification of very low cTn concentrations seen in asymptomatic individuals and the detection of subclinical myocardial damage in individuals without overt acute cardiac disease [8,9]. The term high-sensitivity is defined as the ability to measure troponin concentrations in >50% of a healthy reference population with a coefficient of variation <10% at the 99th percentile [10-12]. Besides the tremendous role in the management of patients with ACS, recent studies demonstrated prognostic implications of hs-cTn determination in individuals without acute cardiac morbidities [13-16]: Hs-cTn is recognized as predictor of all-cause mortality and cardiovascular death in the general population. Currently, there are three hs-cTn assays available. Roche hs-cTnT assay (Roche Diagnostics, Mannheim, Germany) and Abbott hs-cTnI assay (Abbott, Chicago, United States) have been used for several years. Numerous studies focused on diagnostic performance and clinical utility of hs-cTn determination in patients with ACS and in the general population [13-18]. Recently, a novel hs-cTnI assay by Siemens (Siemens Healthineers, Eschborn, Germany), following referred to as ADVIA Centaur hs-cTnI, has become available. In a study by Boeddinghaus and colleagues, this

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3 novel hs-cTnI assay showed high diagnostic accuracy and clinical performance in diagnosing ACS
4 and a comparable performance to the aforementioned hs-cTn assays [8].
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7 Patients with diabetes mellitus show elevated plasma cTn concentrations and various diabetes
8 associated factors contributing to the increased CVD risk including hyperglycemia, dyslipidemia and
9 alterations in the hemostasis system [19-22]. A useful tool to assess early forms of atherosclerosis in
10 clinical practice is the determination of carotid intima-media thickness (cIMT) by high-resolution
11 ultrasound. cIMT increases with age and is known to be larger in patients with T2DM compared to
12 healthy controls [23,24]. While cIMT is a risk marker for future CVD events in the general population
13 [24], cIMT progression was not associated with future cardiovascular risk in patients with diabetes
14 [25].
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16 We now addressed the contribution of hs-cTnI, measured by the novel ADVIA Centaur hs-cTnI assay,
17 to cardiovascular risk which was assessed by cIMT in T2DM. We furthermore compared this to
18 classical clinical CVD risk factors.
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Methods

Cohort

234 patients with T2DM were included in the analysis. 100 of the patients were women (43%) and 134 men (58%) with a median age of 65 years (57-71) and the median duration of diabetes mellitus was 10 years (6-17). Anthropometric data, clinical characteristics and laboratory parameters are shown in Table 1. The study was conducted at the Department of Internal Medicine of the University Hospital Tübingen. Data were obtained from inpatients taking part at a diabetes mellitus education program according to recent guidelines [26]. Patients with type 1 diabetes mellitus and those who had a record of diabetes secondary to other causes (e.g. genetic defects of beta cell function, monogenic forms of diabetes, diabetes mellitus due to other pancreatic diseases) were excluded from the study. A detailed history including demographics and time since diagnosis of T2DM was taken from all subjects, followed by routine laboratory testing and physical examination including anthropometric data acquisition such as body mass index. In addition, patients were asked for history or presence of macrovascular disease (coronary artery disease, peripheral vascular disease, cerebrovascular disease). Blood pressure was measured according to Riva-Rocci by auscultation after resting for 10 minutes in sitting position. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki from 1964 and its later amendments. Written informed consent was obtained from all study participants. The local ethics committee approved the protocol.

Laboratory assessment and clinical parameters

Determination of hs-cTnI was performed on an ADVIA Centaur XPT Immunoassay System (Siemens Healthineers, Eschborn, Germany) using re-thawed lithium-heparinized plasma samples. The limit of detection is indicated according to the manufacturer as 2.21 ng/l, the limit of blank as 0.90 ng/l and the 99th percentiles concentrations are reported as 57 ng/l and 37 ng/l for men and women, respectively. All other clinical chemistry analyses (i.e. creatinine [enzymatic method], glucose [hexokinase method], total cholesterol, high-density lipoprotein-cholesterol [HDL-cholesterol], low-density lipoprotein-cholesterol [LDL-cholesterol], triglycerides and C-reactive protein) were measured on an ADVIA Chemistry XPT analyzer following manufacturer's instructions, except for apolipoprotein AI

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3 and apolipoprotein B, which were measured on a BN ProSpec System (Siemens Healthineers). All
4 laboratory parameters were measured after an overnight fasting period. Glycated hemoglobin (HbA1c)
5 was measured using EDTA whole blood on a Tosoh G8 HPLC Analyzer (Tosoh Bioscience,
6 Griesheim, Germany). Estimated glomerular filtration rate (eGFR) was calculated using a modified
7 MDRD (Modification of Diet in Renal Disease)-formula based on plasma creatinine concentration,
8 gender and age [27].
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18 *Measurement of carotid intima-media thickness*

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20 The cIMT of the common carotid artery was measured using high-resolution ultrasound with a 10-13
21 MHz-linear array transducer (AU5 Harmonic, Esaote Biomedica, Munich, Germany). Ultrasound of
22 both left and right common carotid artery was performed according to the European Mannheim carotid
23 intima-media thickness consensus [28]. In detail, B-mode cIMT was assessed on the far wall of the
24 artery approximately 10 mm proximal to the carotid bulb as described before [29].
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32 *Statistical analysis*

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34 Continuous data were presented as mean \pm standard deviation for normally distributed variables and as
35 median (lower quartile - upper quartile) for non-normally distributed variables. Normal distribution
36 was tested for all continuous variables using the Shapiro-Wilk-W test. Non-normal distributed data
37 were transformed with a logarithmic function prior to statistical analyses. The non-parametric rank
38 sum test was used to compare two groups. The association of hs-cTnI results with clinical and
39 laboratory variables were analysed using simple and multiple linear regression analysis. A p-value
40 <0.05 was considered to indicate significant difference. For the selection of variables in the multiple
41 regression analysis, stepwise linear regression (forward selection) was performed. Statistical analyses
42 were conducted with JMP 14 (SAS Institute, Cary, United States). Figure 1 was created using
43 GraphPad Software (version 8; GraphPad Software Inc., San Diego, United States).
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Results

High-sensitivity cardiac troponin I in patients with type 2 diabetes mellitus

Plasma hs-cTnI concentrations were measurable in 226 patients (97%) with a median hs-cTnI concentration of 4.0 ng/l (2.0-10.0) as presented in Table 1. Among all T2DM patients, 9 individuals (4%) had hs-cTnI concentrations above the 99th gender-specific percentile. 217 individuals (93%) had concentrations below these thresholds and 72 individuals (31%) showed concentrations below the limit of detection (2.21 ng/ml) of which 8 individuals (3%) had concentrations below the limit of blank (0.90 ng/l). Median concentration of hs-cTnI was higher in men compared to women (5.0 ng/l vs. 3.0 ng/l; $p=0.0005$). Total cholesterol (4.5 mmol/l vs. 4.8 mmol/l; $p=0.0048$) and HDL-cholesterol (1.1 mmol/l vs. 1.2 mmol/l; $p=0.0025$) concentrations were lower in men compared to women. In addition, mean concentration of apolipoprotein AI (154 mg/dl vs. 144 mg/dl; $p=0.0002$), median concentrations of apolipoprotein B (99 mg/dl vs. 93 mg/dl; $p=0.0379$) and C-reactive protein (0.45 mg/dl vs. 0.23 mg/dl; $p=0.0030$) were higher in women than in men. Although median creatinine concentration (0.9 mg/dl vs. 0.7 mg/dl; $p<0.0001$) was significantly higher in men compared to women, the calculated eGFR (MDRD) did not differ between genders ($p=0.8781$).

137 patients (59%) used lipid-lowering drugs. Metformin was taken by 156 patients (67%) and 137 patients (59%) were on insulin therapy. 35 patients (15%) reported to be smokers and 75 patients (35%) had a history of smoking. Median cIMT was 0.75 mm, ranging from 0.70-0.95 mm with higher cIMT in men compared to women (0.80 mm vs. 0.73 mm; $p=0.0021$). Macrovascular disease was present in 56 of the men (41%) and in 18 of the women (18%) with T2DM, with a significant difference comparing the presence of macrovascular disease by gender ($p<0.0001$).

Associations between hs-cTnI concentrations and markers of cardiovascular risk and vascular damage

For the understanding of the association of hs-cTnI concentrations with cIMT and traditional cardiovascular risk factors, we performed simple linear regression analyses (Table 2). In the entire cohort, hs-cTnI was significantly associated with age (effect size $\beta_{st}=0.25$; $p=0.0001$), gender ($\beta_{st}=-$

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3 0.24; $p=0.0003$), macrovascular disease ($\beta_{st}=-0.23$; $p=0.0005$), eGFR ($\beta_{st}=-0.29$; $p<0.0001$) and cIMT
4 ($\beta_{st}=0.27$; $p<0.0001$). As cIMT interacted with gender on hs-cTnI ($p=0.0256$), subsequent analyses
5 were performed separately for both genders. In men, significant associations of hs-cTnI with age
6 ($\beta_{st}=0.29$; $p=0.0009$), HDL-cholesterol ($\beta_{st}=0.18$; $p=0.0452$), eGFR ($\beta_{st}=-0.39$; $p<0.0001$) and cIMT
7 ($\beta_{st}=0.34$; $p<0.0001$) were detected in univariate analysis. In women, only age was significantly
8 associated with hs-cTnI ($\beta_{st}=0.24$; $p=0.0178$). CIMT and eGFR showed no significant association with
9 hs-cTnI in women (Table 2 and Figure 1). To identify independent predictors of hs-cTnI multiple
10 linear regression analysis were performed. Therefore, stepwise regression analysis was utilized to
11 identify variables for the multivariable regression model (Table 3). In the final analyses only gender
12 ($\beta_{st}=-0.22$; $p=0.0007$), eGFR ($\beta_{st}=-0.20$; $p=0.0028$) and C-reactive protein ($\beta_{st}=0.14$; $p=0.0234$) were
13 significantly associated with hs-cTnI in the entire cohort. Analysis separated by gender revealed eGFR
14 ($\beta_{st}=-0.24$; $p=0.0126$) and cIMT ($\beta_{st}=0.24$; $p=0.0081$) to be independently associated with hs-cTnI in
15 men (Table 3). Adjusting for the same covariates in women, age ($\beta_{st}=0.24$; $p=0.0280$) remained as a
16 single factor significantly associated with hs-cTnI. Finally, the influence of traditional cardiovascular
17 risk factors, such as body mass index, systolic blood pressure, LDL-cholesterol and smoking on the
18 final model was tested. CIMT ($\beta_{st}=0.25$; $p=0.0067$) and eGFR ($\beta_{st}=-0.23$; $p=0.0169$) still remained
19 significantly associated with hs-cTnI in men and, again, only the association between hs-cTnI and age
20 in women ($\beta_{st}=0.27$; $p=0.0234$).

Discussion

We here investigated links between high-sensitivity cardiac troponin I (hs-cTnI) and cardiovascular risk factors and early signs of arteriosclerotic disease in patients with type 2 diabetes. Our results revealed important gender-specific differences: Men showed higher cIMT compared to women as shown before [29,30]. In men, cIMT was significantly associated with elevated hs-cTnI concentrations, a marker of subclinical myocardial damage, independently of traditional CVD risk factors, such as age, body mass index, smoking and LDL-cholesterol. The association between subclinical myocardial damage and cIMT was independent of the presence of macrovascular disease. Interestingly, women did not show the association between cIMT and hs-cTnI, whereby cIMT gender differences, to the detriment of men, has shown early before [31]. Age was the single independently associated risk factor of hs-cTnI in women with T2DM. This is in line with several other studies reporting age as the main risk factor of cardiovascular death in women [32-34]. A previous study investigated the association of Abbott hs-cTnI and an additional marker of vascular damage by using brachial-arterial pulse wave velocity [35]. A significant association between hs-cTnI and brachial-arterial pulse wave velocity (ba-PWV), a marker of arterial stiffness, was demonstrated, but not with cIMT. Unfortunately, the study did not report gender-specific associations. Nevertheless, their results are in line with our findings in the entire cohort.

T2DM itself is associated with an increased cIMT and gender differences of cIMT are also well documented [23,36-38]. Consequently, gender-specific risk stratification in T2DM should be performed, which may have important implications in prognosis and potentially the treatment of these patients.

Further interesting findings were observed in our study: the majority of T2DM patients in our study showed measurable hs-cTnI concentrations below the gender-specific 99th percentiles. Hs-cTn assays are frequently used today and provide results with strongly increased diagnostic accuracy even at very low troponin concentrations within the reference interval [8]. Several other studies consistently showed that elevated troponin plasma concentrations are present in patients with T2DM patients compared to non-diabetics either using older troponin assays or hs-cTn assays [39,40]. It was shown

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3 that even slightly increased hs-cTn concentrations, still within the reference interval, are associated
4 with subclinical myocardial damage and are therefore predictive for future cardiovascular events in the
5 general population and in patients attending an emergency room without obvious cardiac diseases
6 [13,14,16]. This link emphasizes the need to include hs-cTn assays in the risk stratification of patients
7 with T2DM to identify individuals at increased risk for cardiovascular events.
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11 We found increased hs-cTnI concentrations in men compared to women as it was previously
12 demonstrated with the introduction of the first hs-cTn assays gender-specific differences of hs-cTn
13 concentrations [41,42]. In a recent study, Boeddinghaus and colleagues confirmed gender-specific
14 differences determined by the same hs-cTnI assay we used in our study in patients presenting with
15 suspicion of acute coronary syndrome to the emergency unit [8]. We observed nearly 2-fold higher hs-
16 cTnI in men as it was consistently reported by the aforementioned studies. It is now widely accepted
17 that troponin concentrations, in line with other biomarkers, are influenced by gender and that gender-
18 specific 99th percentiles must be taken into account [5,11].
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22 Renal function, determined by eGFR (MDRD), was found to be another parameter, which is
23 associated with hs-cTnI in our study. And again, this association was only true in men. Similar
24 findings were reported in previous publications showing significant association between renal function
25 and hs-cTn concentrations in patients admitting the emergency room [43,44]. Studies using hs-cTnT
26 and hs-cTnI assays in parallel showed stronger correlation of renal function to hs-cTnT compared to
27 hs-cTnI [43,44]. Unfortunately, we did not measure hs-cTnT in our study to focus on this specific
28 issue. In our study, the majority of T2DM patients had an eGFR within the reference interval. It is
29 therefore not possible to make detailed statements to the association of hs-cTnI and chronic kidney
30 disease, especially, since we do not have an additional marker for early nephropathy such as albumin
31 in urine measurement. However, there is evidence that hs-cTnI is not increased in patients with
32 dialysis-dependent renal failure. In a recent study it was demonstrated that hs-cTnI concentrations
33 were below the 99th percentile in the majority of these patients [45].
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37 Strength of our study is the observation in a real-world clinical setting in a well characterized cohort of
38 patients with T2DM. We assessed subclinical myocardial damage using a novel hs-cTnI assay and
39 investigated the association to cIMT, an established marker of atherosclerosis. Limitations of the study
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3 include the cross-sectional design and the lack of detailed data about cardiac parameters (i.e. cardiac
4 function). Regarding cIMT as an early marker of subclinical atherosclerosis, a point of limitation is
5 that we used this tool in a cohort including patients with known macrovascular disease. Nevertheless,
6 we are looking at cIMT as a continuous variable and an increment of cIMT is associated with an
7 elevated CVD risk [24]. We did not examine more distal segments of the carotid artery (i.e. the
8 internal carotid artery) or plaque burden. Latter is known to be an important marker of advanced
9 atherosclerosis [46]. Regarding laboratory parameters, the MDRD formula for estimating GFR used in
10 this study is not accurate for values within the normal range and results should therefore be carefully
11 interpreted. Finally, results of the study need to be validated in larger cohorts, ideally combined with
12 follow-up measurements to investigate the prognostic utility of the association between hs-cTnI and
13 cIMT.
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28 In conclusion, the present study in a real-world clinical setting demonstrated that the majority of
29 patients with T2DM showed measurable hs-cTnI concentrations using the novel ADIVA centaur hs-
30 cTnI assay. CIMT was significantly associated with hs-cTnI in men and is therefore suitable as
31 predictor of subclinical myocardial damage in men, but not in women.
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FOR PEER REVIEW

References

1. Everett BM, Brooks MM, Vlachos HE et al. Troponin and Cardiac Events in Stable Ischemic Heart Disease and Diabetes. *N Engl J Med* 2015; 373: 610-620 DOI: 10.1056/NEJMoa1415921
2. [Anonymous]. Diabetes mellitus: a major risk factor for cardiovascular disease. A joint editorial statement by the American Diabetes Association; The National Heart, Lung, and Blood Institute; The Juvenile Diabetes Foundation International; The National Institute of Diabetes and Digestive and Kidney Diseases; and The American Heart Association. *Circulation* 1999; 100: 1132-1133
3. Lloyd-Jones DM, Leip EP, Larson MG et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation* 2006; 113: 791-798 DOI: 10.1161/CIRCULATIONAHA.105.548206
4. Emerging Risk Factors C, Sarwar N, Gao P et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; 375: 2215-2222 DOI: 10.1016/S0140-6736(10)60484-9
5. Thygesen K, Alpert JS, Jaffe AS et al. Fourth universal definition of myocardial infarction (2018). *Eur Heart J* 2019; 40: 237-269 DOI: 10.1093/eurheartj/ehy462
6. Thygesen K, Mair J, Katus H et al. Recommendations for the use of cardiac troponin measurement in acute cardiac care. *Eur Heart J* 2010; 31: 2197-2204 DOI: 10.1093/eurheartj/ehq251
7. Damman P, van 't Hof AW, Ten Berg JM et al. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: comments from the Dutch ACS working group. *Neth Heart J* 2017; 25: 181-185 DOI: 10.1007/s12471-016-0939-y
8. Boeddinghaus J, Twerenbold R, Nestelberger T et al. Clinical Validation of a Novel High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction. *Clin Chem* 2018; 64: 1347-1360 DOI: 10.1373/clinchem.2018.286906
9. McCarthy CP, Raber I, Chapman AR et al. Myocardial Injury in the Era of High-Sensitivity Cardiac Troponin Assays: A Practical Approach for Clinicians. *JAMA Cardiol* 2019: DOI: 10.1001/jamacardio.2019.2724
10. Goodman SG, Steg PG, Eagle KA et al. The diagnostic and prognostic impact of the redefinition of acute myocardial infarction: lessons from the Global Registry of Acute Coronary Events (GRACE). *Am Heart J* 2006; 151: 654-660 DOI: 10.1016/j.ahj.2005.05.014
11. Apple FS, Sandoval Y, Jaffe AS et al. Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care. *Clin Chem* 2017; 63: 73-81 DOI: 10.1373/clinchem.2016.255109
12. Frankenstein L, Wu AH, Hallermayer K et al. Biological variation and reference change value of high-sensitivity troponin T in healthy individuals during short and intermediate follow-up periods. *Clin Chem* 2011; 57: 1068-1071 DOI: 10.1373/clinchem.2010.158964
13. Willeit P, Welsh P, Evans JDW et al. High-Sensitivity Cardiac Troponin Concentration and Risk of First-Ever Cardiovascular Outcomes in 154,052 Participants. *J Am Coll Cardiol* 2017; 70: 558-568 DOI: 10.1016/j.jacc.2017.05.062

14. Blankenberg S, Salomaa V, Makarova N et al. Troponin I and cardiovascular risk prediction in the general population: the BiomarCaRE consortium. *Eur Heart J* 2016; 37: 2428-2437 DOI: 10.1093/eurheartj/ehw172
15. Seliger SL, Hong SN, Christenson RH et al. High-Sensitive Cardiac Troponin T as an Early Biochemical Signature for Clinical and Subclinical Heart Failure: MESA (Multi-Ethnic Study of Atherosclerosis). *Circulation* 2017; 135: 1494-1505 DOI: 10.1161/CIRCULATIONAHA.116.025505
16. Zhu K, Knuiman M, Divitini M et al. High-sensitivity cardiac troponin I and risk of cardiovascular disease in an Australian population-based cohort. *Heart* 2018; 104: 895-903 DOI: 10.1136/heartjnl-2017-312093
17. Welsh P, Preiss D, Shah ASV et al. Comparison between High-Sensitivity Cardiac Troponin T and Cardiac Troponin I in a Large General Population Cohort. *Clin Chem* 2018; 64: 1607-1616 DOI: 10.1373/clinchem.2018.292086
18. Rubini Gimenez M, Twerenbold R, Reichlin T et al. Direct comparison of high-sensitivity-cardiac troponin I vs. T for the early diagnosis of acute myocardial infarction. *Eur Heart J* 2014; 35: 2303-2311 DOI: 10.1093/eurheartj/ehu188
19. Hallen J, Johansen OE, Birkeland KI et al. Determinants and prognostic implications of cardiac troponin T measured by a sensitive assay in type 2 diabetes mellitus. *Cardiovasc Diabetol* 2010; 9: 52 DOI: 10.1186/1475-2840-9-52
20. Festa A, D'Agostino R, Jr., Tracy RP et al. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2002; 51: 1131-1137
21. Kannel WB. Lipids, diabetes, and coronary heart disease: insights from the Framingham Study. *Am Heart J* 1985; 110: 1100-1107
22. Balletshofer BM, Rittig K, Enderle MD et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 2000; 101: 1780-1784
23. Brohall G, Oden A, Fagerberg B. Carotid artery intima-media thickness in patients with Type 2 diabetes mellitus and impaired glucose tolerance: a systematic review. *Diabet Med* 2006; 23: 609-616 DOI: 10.1111/j.1464-5491.2005.01725.x
24. Lorenz MW, Markus HS, Bots ML et al. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007; 115: 459-467 DOI: 10.1161/CIRCULATIONAHA.106.628875
25. Lorenz MW, Price JF, Robertson C et al. Carotid intima-media thickness progression and risk of vascular events in people with diabetes: results from the PROG-IMT collaboration. *Diabetes Care* 2015; 38: 1921-1929 DOI: 10.2337/dc14-2732
26. Dallosso H, Yates T, Mani H et al. Movement through Active Personalised engagement (MAP) - a self-management programme designed to promote physical activity in people with multimorbidity: study protocol for a randomised controlled trial. *Trials* 2018; 19: 576 DOI: 10.1186/s13063-018-2939-2
27. Levey AS, Coresh J, Greene T et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* 2007; 53: 766-772 DOI: 10.1373/clinchem.2006.077180

- 1
2
3 28. Touboul PJ, Hennerici MG, Meairs S et al. Mannheim carotid intima-media thickness and
4 plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th
5 and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences,
6 Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011.
7 *Cerebrovasc Dis* 2012; 34: 290-296 DOI: 10.1159/000343145
8
- 9 29. Randrianarisoa E, Rietig R, Jacob S et al. Normal values for intima-media thickness of the
10 common carotid artery--an update following a novel risk factor profiling. *Vasa* 2015; 44: 444-
11 450 DOI: 10.1024/0301-1526/a000467
12
- 13 30. Chambless LE, Folsom AR, Davis V et al. Risk factors for progression of common carotid
14 atherosclerosis: the Atherosclerosis Risk in Communities Study, 1987-1998. *Am J Epidemiol*
15 2002; 155: 38-47
16
- 17 31. O'Leary DH, Polak JF, Kronmal RA et al. Distribution and correlates of sonographically
18 detected carotid artery disease in the Cardiovascular Health Study. The CHS Collaborative
19 Research Group. *Stroke* 1992; 23: 1752-1760
20
- 21 32. Mosca L, Benjamin EJ, Berra K et al. Effectiveness-based guidelines for the prevention of
22 cardiovascular disease in women--2011 update: a guideline from the american heart
23 association. *Circulation* 2011; 123: 1243-1262 DOI: 10.1161/CIR.0b013e31820faaf8
24
- 25 33. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause
26 and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the
27 Global Burden of Disease Study 2013. *Lancet* 2015; 385: 117-171 DOI: 10.1016/S0140-
28 6736(14)61682-2
29
- 30 34. Maffei S, Guiducci L, Cugusi L et al. Women-specific predictors of cardiovascular disease
31 risk - new paradigms. *Int J Cardiol* 2019: DOI: 10.1016/j.ijcard.2019.02.005
32
- 33 35. Yiu KH, Zhao CT, Chen Y et al. Association of subclinical myocardial injury with arterial
34 stiffness in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol* 2013; 12: 94 DOI:
35 10.1186/1475-2840-12-94
36
- 37 36. Wagenknecht LE, D'Agostino RB, Jr., Haffner SM et al. Impaired glucose tolerance, type 2
38 diabetes, and carotid wall thickness: the Insulin Resistance Atherosclerosis Study. *Diabetes*
39 *Care* 1998; 21: 1812-1818
40
- 41 37. Temelkova-Kurktschiev TS, Koehler C, Leonhardt W et al. Increased intimal-medial thickness
42 in newly detected type 2 diabetes: risk factors. *Diabetes Care* 1999; 22: 333-338
43
- 44 38. Kablak-Ziembicka A, Przewlocki T, Tracz W et al. Gender differences in carotid intima-
45 media thickness in patients with suspected coronary artery disease. *Am J Cardiol* 2005; 96:
46 1217-1222 DOI: 10.1016/j.amjcard.2005.06.059
47
- 48 39. Segre CA, Hueb W, Garcia RM et al. Troponin in diabetic patients with and without chronic
49 coronary artery disease. *BMC Cardiovasc Disord* 2015; 15: 72 DOI: 10.1186/s12872-015-
50 0051-z
51
- 52 40. Cavender MA, White WB, Jarolim P et al. Serial Measurement of High-Sensitivity Troponin I
53 and Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus in the EXAMINE
54 Trial (Examination of Cardiovascular Outcomes With Alogliptin Versus Standard of Care).
55 *Circulation* 2017; 135: 1911-1921 DOI: 10.1161/CIRCULATIONAHA.116.024632
56
57
58
59
60

- 1
2
3 41. Collinson PO, Heung YM, Gaze D et al. Influence of population selection on the 99th
4 percentile reference value for cardiac troponin assays. *Clin Chem* 2012; 58: 219-225 DOI:
5 10.1373/clinchem.2011.171082
6
7 42. Apple FS, Simpson PA, Murakami MM. Defining the serum 99th percentile in a normal
8 reference population measured by a high-sensitivity cardiac troponin I assay. *Clin Biochem*
9 2010; 43: 1034-1036 DOI: 10.1016/j.clinbiochem.2010.05.014
10
11 43. Guclu T, Bolat S, Senes M et al. Relationship between high sensitivity troponins and
12 estimated glomerular filtration rate. *Clin Biochem* 2016; 49: 467-471 DOI:
13 10.1016/j.clinbiochem.2015.12.012
14
15 44. Twerenbold R, Wildi K, Jaeger C et al. Optimal Cutoff Levels of More Sensitive Cardiac
16 Troponin Assays for the Early Diagnosis of Myocardial Infarction in Patients With Renal
17 Dysfunction. *Circulation* 2015; 131: 2041-2050 DOI:
18 10.1161/CIRCULATIONAHA.114.014245
19
20 45. Artunc F, Haag S, Friedrich B et al. Performance of a novel high sensitivity cardiac troponin I
21 assay in asymptomatic hemodialysis patients - evidence for sex-specific differences. *Clin*
22 *Chem Lab Med* 2019; 57: 1261-1270 DOI: 10.1515/cclm-2018-1176
23
24 46. Simon A, Megnien JL, Chironi G. The value of carotid intima-media thickness for predicting
25 cardiovascular risk. *Arterioscler Thromb Vasc Biol* 2010; 30: 182-185 DOI:
26 10.1161/ATVBAHA.109.196980
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Tables and Figures

Figure legend:

Figure 1: Association of high-sensitivity cardiac troponin I (hs-cTnI) with carotid intima media thickness in type 2 diabetes. Shown are results of linear regression and correlation analyses separated by gender. A vertical line indicates the gender-specific 99th percentile cut-off concentration for hs-cTnI.

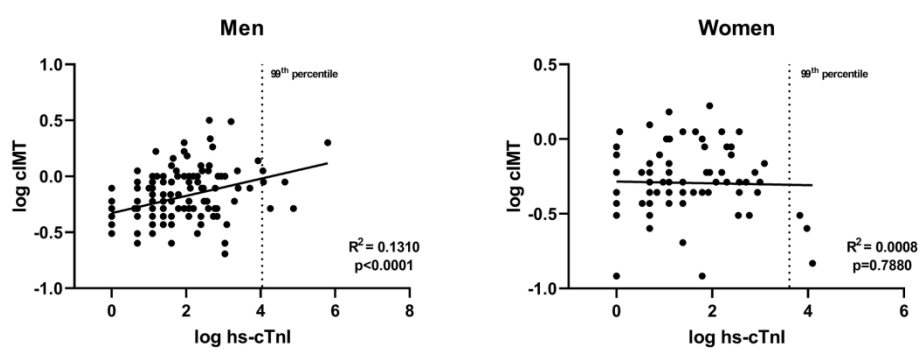
Table legend:

Table 1: Patients' clinical and laboratory characteristics.

Table 2: Predictors of high-sensitivity cardiac troponin I (hs-cTnI) in patients with type 2 diabetes. Shown are effect sizes (β_{st}) and p-values of univariate relationships between hs-cTnI and anthropometric or laboratory parameters in the entire cohort and separated by gender.

Table 3: Multiple linear regression analysis of high-sensitivity cardiac troponin I with clinical and laboratory variables in patients with type 2 diabetes. Shown are effect sizes (β_{st}) and p-values of multivariable regression analysis with all indicated variables.

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Table 1: Patients' clinical and laboratory characteristics (n=234).

Variable	Total cohort (n=234)	Men (n=134)	Women (n=100)	p-value men vs. women
Age, years	65 (57 – 71)	65 (57 – 71)	64 (56 – 71)	0.9566
Body-Mass-Index, kg/m ²	32.8 (28.4 – 39.0)	33.3 (29.0 – 38.8)	31.4 (27.0 – 39.3)	0.1070
Time since diabetes diagnosis, years	10 (6 – 17)	11 (6 – 16)	9 (5 – 18)	0.7619
Systolic blood pressure, mmHg	130 (122 – 140)	130 (120 – 140)	130 (125 – 140)	0.1546
Diastolic blood pressure, mmHg	80 (75 – 80)	80 (75 – 80)	80 (75 – 82)	0.3778
Glucose (fasting), mmol/l	8.6 (7.2 – 10.6)	8.6 (7.2 – 11.0)	8.4 (6.9 – 10.0)	0.1626
Glycated haemoglobin (HbA1c), %	7.6 (6.9 – 9.1)	7.7 (6.9 – 9.2)	7.6 (6.8 – 8.9)	0.3062
Glycated haemoglobin (HbA1c), mmol/mol	59.6 (51.9 – 76.0)	60.7 (51.9 – 76.0)	59.6 (50.8 – 73.8)	
Creatinine, mg/dl	0.8 (0.7 – 1.0)	0.9 (0.8 – 1.2)	0.7 (0.6 – 0.9)	<.0001
eGFR (MDRD), ml/min/1.73 m ²	84 (61 – 100)	84 (60 – 99)	83 (62 – 102)	0.8781
Triglycerides, mmol/l	1.9 (1.4 – 2.4)	1.9 (1.3 – 2.2)	1.9 (1.4 – 2.7)	0.5163
Total cholesterol, mmol/l	4.6 (4.0 – 5.3)	4.5 (3.8 – 5.2)	4.8 (4.1 – 5.5)	0.0048
HDL-Cholesterol, mmol/l	1.1 (0.9 – 1.3)	1.1 (0.9 – 1.3)	1.2 (1.0 – 1.4)	0.0029
LDL-Cholesterol, mmol/l	3.0 (2.4 – 3.9)	3.0 (2.3 – 3.8)	3.0 (2.6 – 4.1)	0.0523
Apolipoprotein AI, mg/dl	149 ± 22	144 ± 20	154 ± 22	0.0002
Apolipoprotein B, mg/dl	95 (80 – 112)	93 (77 – 110)	99 (82 – 118)	0.0379
Apolipoprotein B/Apolipoprotein AI	0.6 (0.5 – 0.8)	0.6 (0.5 – 0.8)	0.6 (0.5 – 0.8)	0.9065
C-reactive Protein, mg/dl	0.33 (0.09 – 0.67)	0.23 (0.07 – 0.62)	0.45 (0.16 – 0.91)	0.0030
Macroangiopathy [#] , % (n)	31% (74)	41% (56)	18% (18)	<.0001
Smoking, % (n)	15% (35)	16% (21)	14% (14)	0.7405
Carotid intima-media thickness, mm	0.75 (0.70 – 0.95)	0.80 (0.70 – 0.95)	0.73 (0.65 – 0.85)	0.0021
High-sensitivity cardiac troponin I*, ng/l	4.0 (2.0 – 10.0)	5.0 (3.0 – 12.0)	3.0 (2.0 – 6.8)	0.0005

[#]Macroangiopathy: evidence for coronary artery syndrome, cerebrovascular disease or peripheral arterial disease

*measurable in 226 patients

eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein

Table 2: Predictors of high-sensitivity cardiac troponin I in patients with type 2 diabetes. Shown are effect sizes (β_{st}) and p-values of univariate relationships between high-sensitivity cardiac troponin I and anthropometric or laboratory parameters in the entire cohort and separated by gender.

Variable	High-sensitivity cardiac troponin I		
	Total cohort (n=226)	Men (n=130)	Women (n=96)
Gender (female = 0, male =1)	-0.24 p=0.0003	-	-
Age, years	0.25 p=0.0001	0.29 p=0.0009	0.24 p=0.0178
Body-Mass-Index, kg/m ²	-0.01 p=0.8925	-0.12 p=0.1831	0.09 p=0.4050
Time since diabetes diagnosis, years	0.06 p=0.3872	0.05 p=0.5381	0.07 p=0.4736
Systolic blood pressure, mmHg	0.03 p=0.6275	0.02 p=0.7816	0.10 p=0.3372
Diastolic blood pressure, mmHg	-0.07 p=0.3192	-0.07 p=0.4054	-0.01 p=0.9066
Glucose (fasting), mmol/l	-0.05 p=0.4953	-0.05 p=0.5508	-0.08 p=0.4375
Glycated haemoglobin, %	-0.09 p=0.1980	-0.09 p=0.2991	-0.12 p=0.2399
eGFR (MDRD), ml/min/1,73 m ²	-0.29 p<.0001	-0.39 p<.0001	-0.18 p=0.0735
Triglycerides, mmol/l	0.01 p=0.9988	-0.02 p=0.8433	0.04 p=0.6832
Total cholesterol, mmol/l	-0.03 p=0.6199	0.02 p=0.8621	-0.01 p=0.8952
HDL-Cholesterol, mmol/l	0.03 p=0.6137	0.18 p=0.0452	-0.03 p=0.7792
LDL-Cholesterol, mmol/l	-0.02 p=0.7448	0.02 p=0.8377	-0.02 p=0.8672
Macrovascular disease (yes = 1)	-0.23 p=0.0005	-0.24 p=0.0050	-0.07 p=0.4993
Smoking (yes = 1)	0.09 p=0.6156	-0.07 p=0.6959	0.17 p=0.7896
C-reactive Protein, mg/dl	0.09 p=0.1561	0.17 p=0.0531	0.08 p=0.4329
Carotid intima-media thickness, mm	0.27 p<.0001	0.34 p<.0001	0.05 p=0.6142

Table 3: Multiple linear regression analysis of associations with high-sensitivity cardiac troponin I in patients with type 2 diabetes. Shown are effect sizes (β_{St}) and p-values of multivariable regression analyses with all indicated variables.

Variable	High-sensitivity cardiac troponin I		
	Total cohort (n=226)	Men (n=130)	Women (n=96)
Gender (female = 0, male =1)	-0.22 0.0007	-	-
Age, years	0.11 0.1064	0.04 0.7094	0.24 0.0280
Glycated haemoglobin, mmol/mol	-0.12 0.0619	-0.10 0.2384	-0.18 0.0927
eGFR, ml/min/1,73 m ²	-0.20 0.0028	-0.24 0.0126	-0.14 0.1764
Macrovascular disease (yes = 1)	-0.09 0.1957	-0.07 0.4142	-0.06 0.5490
C-reactive Protein	0.14 0.0234	0.12 0.1317	0.17 0.1176
Carotid intima-media thickness, mm	0.12 0.0837	0.24 0.0081	-0.09 0.3850