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ORIGINAL ARTICLE

Increased serum S100A12 levels are associated with higher risk of acute heart failure in patients with type 2 diabetes

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Abstract

Aims The hyperglycaemic stress induces the release of inflammatory proteins such as S100A12, one of the endogenous ligands of the receptors for advanced glycation end products (RAGE). Chronic activation of RAGE has multiple deleterious effects in target tissues such as the heart and the vessels by promoting oxidative stress, inflammation by the release of cytokines, macrophages infiltration, and vascular cell migration and proliferation, causing ultimately endothelial cell and cardiomyocyte dysfunction. The aim of our study was to investigate the prognostic value of circulating S100A12 beyond established cardiovascular risk factors (CVRF) for heart failure (HF) and major adverse cardiovascular events (MACE) in a cohort of patients with type 2 diabetes.

Methods and results Serum S100A12 concentrations were measured at baseline in 1345 type 2 diabetes patients (58% men, 64 ± 11 years) recruited in the SURDIAGENE prospective cohort. Endpoints were the occurrence of acute HF requiring hospitalization (HHF) and MACE. We used a proportional hazard model adjusted for established CVRF (age, sex, duration of diabetes, estimated glomerular filtration rate, albumin/creatinine ratio, history of coronary artery disease) and serum S100A12. During the median follow-up of 84 months, 210 (16%) and 505 (38%) patients developed HHF and MACE, respectively. Baseline serum S100A12 concentrations were associated with an increased risk of HHF [hazard ratio (HR) (95% confidence interval) 1.28 (1.01–1.62)], but not MACE [1.04 (0.90–1.20)]. After adjustment for CVRF, S100A12 concentrations remained significantly associated with an increased risk of HHF [1.29 (1.01–1.65)]. In a sub-analysis, patients with high probability of pre-existing HF [N terminal pro brain natriuretic peptide (NT-proBNP) $>$1000 pg/mL, n = 87] were excluded. In the remaining 1258 patients, the association of serum S100A12 with the risk of HHF tended to be more pronounced [1.39 (1.06–1.83)]. When including the gold standard HF marker NT-proBNP in the model, the prognostic value of S100A12 for HHF did not reach significance. Youden method performed at 7 years for HHF prediction yielded an optimal cut-off for S100A12 concentration of 49 ng/mL (sensitivity 53.3, specificity 52.2). Compared with those with S100A12 $\leq$ 49 ng/mL, patients with S100A12 $>$ 49 ng/mL had a significantly increased risk of HHF in the univariate model [HR = 1.58 (1.19–2.09), P = 0.0015] but also in the multivariate model [HR = 1.63 (1.23–2.16), P = 0.0008]. After addition of NT-proBNP to the multivariate model, S100A12 $>$ 49 ng/mL remained associated with an increased risk of HHF [HR = 1.42 (1.07–1.90), P = 0.0160]. However, the addition of S100A12 categories on top of multivariate model enriched by NT-pro BNP did not improve the ability of the model to predict HHF (relative integrated discrimination improvement = 1.9%, P = 0.1500).

Conclusions In patients with type 2 diabetes, increased serum S100A12 concentration is independently associated with risk of HHF, but not with risk of MACE. Compared with NT-proBNP, the potential clinical interest of S100A12 for the prediction of HF events remains limited. However, S100A12 could be a candidate for a multimarker approach for HF risk assessment in diabetic patients.

Keywords S100A12; EN-RAGE; Hospitalization for heart failure; Major adverse cardiovascular events; Type 2 diabetes; Inflammation

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Introduction

Cardiovascular (CV) complications represent the leading cause of morbidity and mortality in patients with type 2 diabetes. Chronic low-grade inflammation is a key process promoting macro-vascular and micro-vascular dysfunctions and driving diabetic CV disease, including heart failure (HF).[1] Recent clinical trials such as LoDoCo[2] and COLCOT[3] provided evidence that inflammation plays a causal role in the occurrence of CV events in patients with chronic or recent CV disease (CVD), respectively, by showing that the anti-inflammatory drug colchicine decreased the relative risk of CV events. The pro-inflammatory and chemoattractant protein S100A12, is released by neutrophils[4] and has been proposed to contribute to multiple chronic inflammatory and autoimmune diseases,[4] and to CVD.[5] S100A12 (also called EN-RAGE, Extracellular Newly identified-RAGE) is a member of S100/calgranulins family and is one of the ligands of RAGEs, the receptors of advanced glycation end products.[4]

Activation of RAGE by its ligands has multiple deleterious effects in target tissues such as the heart and the vessels: It promotes oxidative stress, inflammation by activation of the transcription factor nuclear factor kappa B inducing the release of cytokines (such as TNFα and IL-6) and adhesion molecules, stimulates leukocytes recruitment, macrophages infiltration into the vascular wall, and vascular smooth muscle cell migration and proliferation, causing ultimately endothelial cell and cardiomyocyte dysfunction.[6–8] S100A12 may contribute to atherosclerotic plaque instability[9] and elevated serum S100A12 has a predictive value for plaque rupture in patients with coronary artery disease.[10,11] In addition, high S100A12 concentrations are associated with the occurrence of CV events in patients with chronic HF,[12] with the progression of carotid intima-media-thickness and arterial calcification in haemodialyzed patients.[13–15] More globally, the addition of serum S100A12 to the Framingham risk model improves prediction of adverse outcomes.[16,17] In transgenic mice, S100A12 overexpression directly contributes to left ventricular hypertrophy, aortic valve stenosis, and HF as a result of fibroblast growth factor 23 produced by cardiac fibroblasts in response to the inflammatory milieu mediated by S100A12.[7,18] In the context of hyperglycaemia, S100A12 blood concentrations correlate with biomarkers of diabetes such as HbA_{1C} and fasting glucose concentrations.[19] In addition, in post-mortem coronary artery lesions, Burke *et al*. observed that S100A12 expression was higher in coronary patients with diabetes when compared with those without diabetes.[20]

Despite these data highlighting the association between elevated S100A12 and CVDs on the one hand, and increased S100A12 in diabetes on the other hand, only few studies focused on the association between circulating S100A12 and CV risk in people with diabetes.[21–23] In the present study, we aimed to test in a prospective cohort of patients with type 2 diabetes[24,25] the hypothesis that increased serum S100A12 concentrations are associated with future acute HF events and major adverse CV events (MACE).

Material and methods

Study patients

Participants from the SURDIAGENE cohort were recruited at the University Hospital of Poitiers, France, from 2002 to 2012.[25] Our study complies with the Declaration of Helsinki, the local ethics committee (CPP Ouest III, protocol #03.10.19) approved the study design and written informed consent was obtained from all participants. Participants were prospectively followed-up until death, or up to 31 December 2015.

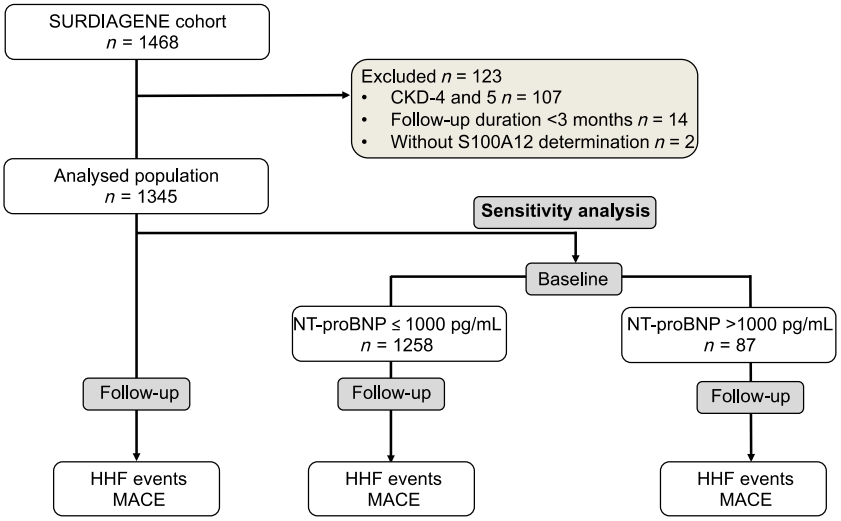
Participants with a baseline estimated glomerular filtration rate (eGFR) <30 mL/min^1/1.73 m^2 and/or a history of prior renal replacement therapy and/or a follow-up duration of <3 months were excluded from the present analysis (*Figure 1*). Demographic and clinical data, morphometric measurements, electrocardiogram, and biological variables were obtained at baseline. History of myocardial infarction or symptomatic peripheral artery disease was self-reported.

Because history of chronic HF was not systematically assessed at inclusion in the SURDIAGENE cohort, we considered elevated baseline NT-proBNP concentrations >1000 pg/mL as a surrogate for high probability of pre-existing chronic HF[26,27] (*Figure 1*).

Definition of outcomes

The primary endpoint was hospitalization for HF (HHF). The secondary endpoint was a composite of non-fatal myocardial infarction, non-fatal stroke, and CV-death (MACE). These clinical questions were raised *post-hoc*. CV endpoints were determined from patients' hospital records and interviews with their general practitioners. Each endpoint was reviewed by an independent adjudication committee according to the international definitions of clinical outcomes. We defined his-

Figure 1 Flow chart. A total of *n* = 1345 patients from the SURDIAGENE cohort were analysed and followed in this prospective study. A sensitivity analysis was performed in patients with baseline NT-proBNP levels ≤1000 pg/mL. CKD, chronic kidney disease; HHF, hospitalization for heart failure; MACE, major adverse cardiovascular events; NT-proBNP, N terminal pro brain natriuretic peptide.



tory of CVD by history of myocardial infarction and/or stroke at baseline.

Assays

Blood samples and second morning urine samples were obtained in individuals at baseline after an overnight fast. HbA1c and serum creatinine concentrations were centrally determined using a chromatography method (ADAMS A1c HA-8160 analyser; Menarini, Florence, Italy) and a colorimetric method on an automated analyser (KONE Optima; Thermo Clinical Labsystems, Vantaa, Finland), respectively. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.[28] Urinary creatinine was measured on a Hitachi 911 automatic analyser (Roche Diagnostics, Meylan, France), and urine albumin by nephelometry on a Modular System P (Roche Diagnostics).

Blood samples were processed under standardized conditions and stored at −80°C in the Poitiers Biological Resource Center (BRC BB-0033-00068, Poitiers, France). High sensitive C-reactive protein (hs-CRP) was measured at baseline using an immunoturbidimetric assay (Roche/Hitachi COBAS C Systems; Roche Diagnostics). Serum concentrations of TNF receptor 1 (TNFR1) were measured using a human soluble TNFR1 ELISA kit (EKF Diagnostics, Dublin, Ireland). Plasma NT-proBNP concentration was measured in a COBAS system by an automated electrochemiluminescence immunoassay according to the manufacturer's information.

Serum S100A12 concentrations were measured using an ELISA kit for human S100A12 (catalogue no. CY-8058 V2; MBL Circulex, Japan), according to the manufacturer's instructions. The limit of detection was 8 pg/mL, the intra-assay coefficient of variability was 3.4–5.3%, and the inter-assay coefficient of variability was 5.3–6.2%. Serum was diluted 1:100 in all samples.

Statistical analysis

Qualitative variables are reported as absolute values and percentages, while quantitative variables are described by mean ± SD or median (25th–75th percentiles) for skewed distributions, when appropriate. Associations between qualitative variables were evaluated using the χ^2 test. Quantitative variables were compared using the Student's *t* test or Mann–Whitney *U* test, as appropriate.

The correlation between two quantitative variables was tested using a Spearman's test. Survival curves were estimated by Kaplan–Meier method and compared with log rank test.

Cox proportional hazard models were used to analyse the effect on study outcomes. Quantitative variables were log-transformed if appropriate in order to meet the Cox model assumptions. Hazard ratio (HR) and their 95% confidence interval (CI) are presented for an increment of one unit or one $\log_{10}$ when appropriate. Models were adjusted for established risk factors (age, sex, duration of diabetes, history of CVD, eGFR, and urine albumin-to-creatinine ratio, as described for MR-proADM,[24] a biomarker of HF previously identified in this cohort.

The relative integrated discrimination improvement (rIDI) index was calculated to assess the improvement in 7 year risk prediction of S100A12 in addition to the top the established risk factors. Seven-year risk was selected because it approximates the median follow-up time for HHF.

We conducted ~~3~~2 sensitivity analyses to investigate (1) whether the S100A12 prognostic value was modified according to prior HF history after exclusion of patients with high probability of pre-existing HF based on baseline NT-proBNP >1000 pg/mL (subgroups analysis), (2) whether NT-proBNP concentration (as covariate) modified our findings, and (3) if we could evidence a potential S100A12 cut-off for the HHF prediction by the Youden index.

P values <0.05 were considered statistically significant. Statistical analyses were carried out using the SAS version 9.4 software package (SAS, Cary, NC, USA) and GraphPad Prism software version 9.1 (San Diego, CA, USA).

Results

Baseline characteristics

Median (25th, 75th) serum S100A12 concentration was 55.1 (27.0, 154.8) ng/mL at baseline. During a median follow-up of 84 (52, 128) months, 210 (16%) HHF events and 505 (38%) MACE occurred. Clinical characteristics of the 1345 patients are summarized in *Table 1*.

Youden method performed at 7 years for HHF prediction retrieved an optimal cut-off for S100A12 concentration of 49 ng/mL (sensitivity 53.3, specificity 52.2). The association of the S100A12 categories with HHF event-free survival is illustrated by Kaplan–Meier curves (*Figure 2*).

S100A12, hospitalization for heart failure, and major adverse cardiovascular events

In univariate analysis, higher concentrations of S100A12 were significantly associated with an increased risk of HHF [HR (95% CI) = 1.28 (1.01–1.62), *P* = 0.0436], but not MACE [HR (95% CI) = 1.04 (0.90–1.20), *P* = 0.6000].

After adjustment for traditional CV risk factors, increase in baseline S100A12 concentrations was associated with a significant higher risk of HHF [HR (95% CI) = 1.29 (1.01–1.65), *P* = 0.0431 (*Figure 2* and *Table 2*)], but not with MACE [HR 0.99 (0.85–1.15), *P* = 0.8849] (*Table 2*).

Compared with those with S100A12 ≤ 49 ng/mL, patients with S100A12 > 49 ng/mL had a statistically significant increase risk of HHF in the univariate model [HR = 1.58 (1.19–2.09), *P* = 0.0015], but also in the multivariate model

Table 1 Clinical characteristics

	All (*n* = 1345)	No HHF during follow-up (*n* = 1135)	HHF during follow-up (*n* = 210)	*P*
Sex, men, *n* (%)	775 (58%)	654 (58%)	121 (58%)	0.9995
Age, years	64 ± 11	63 ± 11	70 ± 9	<0.0001
BMI, kg/m^2	31.4 ± 6.3	31.6 ± 6.3	30.3 ± 5.9	0.0065
Systolic blood pressure, mmHg[a]	132 ± 17	131 ± 17	135 ± 20	0.0156
Diastolic blood pressure, mmHg[a]	73 ± 11	73 ± 11	71 ± 12	0.0825
Diabetes duration, years[a]	14 ± 10	13 ± 9	19 ± 10	<0.0001
HbA1c, %[a]	7.8 ± 1.5	7.8 ± 1.6	7.9 ± 1.4	0.5920
HbA1c, mmol/mol*	62 ± 12	62 ± 13	63 ± 11	
Serum creatinine, μmol/L	81 (68–97)	80 (67–94)	90 (75–112)	<0.0001
eGFR, mL/min^1/1.73 m^2	77 ± 21	79 ± 20	66 ± 20	<0.0001
ACR, mg/mmol[a]	2.7 (1.0, 10.7)	2.4 (0.9, 8.6)	7.4 (1.6, 32.8)	<0.0001
Total cholesterol, mmol/L	4.8 ± 1.1	4.8 ± 1.1	4.8 ± 1.1	0.8257
Active smoking[a], *n* (%)	148 (11%)	136 (12%)	12 (6%)	0.0078
History of coronary heart disease, *n* (%)	349 (26%)	248 (22%)	101 (48%)	<0.0001
History of coronary artery revascularization, *n* (%)	200 (15%)	145 (13%)	55 (26%)	<0.0001
Sinus rhythm[a], *n* (%)	1273 (95%)	1087 (96%)	186 (89%)	<0.0001
hs-CRP[a], mg/L	3.08 (1.37, 6.94)	3.02 (1.32, 6.76)	3.80 (1.82, 9.45)	0.0029
sTNFR1[a], pg/mL	1816 (1544, 2239)	1769 (1528, 2162)	2116 (1700, 2726)	<0.0001
NT-proBNP[a], pg/mL	102 (47–262)	83 (41–203)	337 (160–832)	<0.0001
S100A12, ng/mL	55 (27–155)	54 (27–143)	60 (30–212)	0.1038
ACE inhibitors	500 (37%)	393 (35%)	107 (51%)	<0.0001
AT2-blockers	376 (28%)	320 (28%)	56 (27%)	0.6505
β-blockers	453 (34%)	355 (31%)	98 (47%)	<0.0001
Diuretics	601 (45%)	472 (42%)	129 (61%)	<0.0001

ACE, angiotensin-converting enzyme; ACR, albumin-to-creatinine ratio; CRP, C-reactive protein; HHF, hospitalization for heart failure; hs-CRP, high sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; NT-proBNP, N terminal pro brain natriuretic peptide.
[a]Missing data: Systolic/diastolic blood pressure: 7; diabetes duration: 2; HbA1c: 1; ACR: 4, smoking status: 17; sinus rhythm: 6; hs-CRP: 81; sTNFR1: 3; NT-proBNP: 5.

Figure 2 Kaplan–Meier hospitalization for heart failure (HHF)-free survival curves according to the S100A12 categories. Youden method performed at 7 years for HHF prediction retrieved an optimal cut-off for S100A12 concentration of 49 ng/mL (sensitivity 53.3, specificity 52.2).

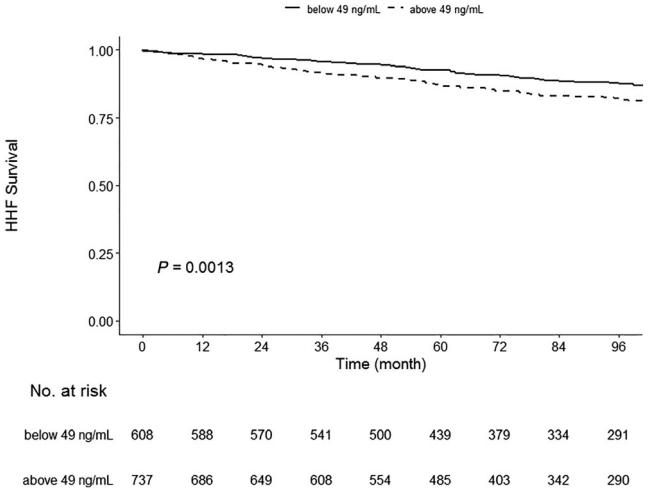


Table 2 Cox multivariate analysis for the risk of HHF and MACE

	HR	95% CI	*P* value
HHF			
Age (per year)	1.06	(1.04–1.08)	<0.0001
Sex (ref. M)	0.99	(0.74–1.31)	0.9283
Duration of diabetes (per year)	1.02	(1.01–1.04)	0.0003
eGFR (per 10 mL/min^{1}/1.73 m^{2})	0.95	(0.87–1.03)	0.1926
ACR (per $\log_{10}$)	1.63	(1.36–1.95)	<0.0001
History of coronary heart disease	2.45	(1.85–3.24)	<0.0001
S100A12 (per $\log_{10}$)	1.29	(1.01–1.65)	0.0422
MACE			
Age (per year)	1.06	(1.04–1.07)	<0.0001
Sex (ref. M)	0.70	(0.58–0.84)	0.0002
Duration of diabetes (per year)	1.01	(1.00–1.02)	0.0648
eGFR (per 10 mL/min^{1}/1.73 m^{2})	0.98	(0.93–1.03)	0.3847
ACR (per $\log_{10}$)	1.59	(1.42–1.78)	<0.0001
History of coronary heart disease	1.50	(1.24–1.81)	<0.0001
S100A12 (per $\log_{10}$)	0.99	(0.85–1.15)	0.8867

ACR, albumin-to-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HHF, hospitalization for heart failure; HR, hazard ratio; MACE, major adverse cardiovascular events.

[HR = 1.63 (1.23–2.16), *P* = 0.0008]. After addition of NT-proBNP to the multivariate model, S100A12 > 49 ng/mL remained associated with an increased risk of HHF [HR = 1.42 (1.07–1.90), *P* = 0.0160]. However, the addition of S100A12 categories on top of the multivariate model enriched by NT-pro BNP did not improve the ability of the model to predict HHF (rIDI = 1.9%, *P* = 0.1500).

In a subgroup analysis focusing on patients with low-to-intermediate likelihood of CHF at inclusion as suggested by baseline NT-proBNP concentration of ≤1000 pg/mL (*N* = 1258), 167 patients (13%) were hospitalized for HF during the follow-up (*Table 3*). These patients were older, had higher systolic blood pressure, longer diabetes duration, more impaired renal function, more frequently history of CAD and coronary revascularization, higher baseline concentrations of the inflammatory markers (hs-CRP and TNFR1), and higher concentrations of NT-proBNP (*Table 3*). In multivariate analysis, increased baseline S100A12 concentrations were significantly associated with an increased risk of HHF [HR 1.39 (1.06–1.83), *P* = 0.0171 (*Figure 3* and *Table 4*)]. In contrast, after inclusion of the gold standard HF marker NT-proBNP—which was weakly correlated with S100A12 (Rho = 0.07)—in the models, the prognostic value of S100A12 for HHF did not reach significance neither in univariate analysis [HR = 1.13 (0.88–1.45) *P* = 0.3323], nor in multivariate analysis [HR = 1.17 (0.91–1.51) *P* = 0.2267]. Thus, NT-proBNP remains the gold standard HF biomarker.

Of note, in the sub-group of patients with NT-proBNP >1000 pg/mL, concentrations of S100A12 were significantly higher when compared with those with NT-proBNP ≤1000 pg/mL [83 ng/mL (39–231), *n* = 87 vs. 54 ng/mL (26–150), *n* = 1258, *P* = 0.0204], supporting the association of S100A12 levels with HHF. In these latter patients, who likely display HF at inclusion, S100A12 has no incremental prognostic value for future HHF [HR = 0.87 (0.43–1.78), *Figure 3*].

Discussion

The main finding of the present study is that in patients with type 2 diabetes, serum S100A12 concentration is independently associated with an increased risk of HHF during the follow-up, but not with MACE. This association tended to be more pronounced after exclusion of patients with high probability of pre-existing CHF at inclusion, with an increased risk of HHF of nearby 40%. Even in patients with three-digit NT-proBNP levels at inclusion, S100A12 has no incremental

Table 3 Clinical characteristics of patients with NT-proBNP ≤1000 pg/mL

	All (n = 1258)	No HHF during follow up (n = 1091)	HHF during follow up (n = 167)	P
Sex, men, n (%)	718 (57%)	625 (57%)	93 (56%)	0.6976
Age, years	64 ± 11	63 ± 10	70 ± 9	<0.0001
BMI, kg/m^2	31.6 ± 6.3	31.7 ± 6.3	30.9 ± 6.0	0.1257
Systolic BP, mmHg[a]	132 ± 17	131 ± 17	136 ± 18	0.0010
Diastolic BP, mmHg[a]	73 ± 11	73 ± 11	72 ± 13	0.2150
Diabetes duration, year[a]	14 ± 10	13 ± 9	19 ± 10	<0.0001
HbA1c, %[a]	7.8 ± 1.6	7.8 ± 1.6	7.9 ± 1.4	0.3118
HbA1c, mmol/mol	62 ± 12	62 ± 12	63 ± 11	
Serum creatinine, μmol/l	80 (68–94)	79 (67–93)	86 (72–105)	<0.0001
eGFR, mL/min^1/1.73 m^2	78 ± 20	79 ± 20	69 ± 20	<0.0001
uACR, mg/mmol[a]	2.5 (1.0–9.6)	2.3 (0.9–8.0)	7.5 (1.5–32.8)	<0.0001
Total cholesterol, mmol/L	4.8 ± 1.1	4.8 ± 1.1	4.8 ± 1.1	0.5690
Active smoking[a], n (%)	146 (12%)	134 (12%)	12 (7%)	0.0553
History of coronary heart disease, n (%)	956 (76%)	225 (21%)	77 (46%)	<0.0001
History of coronary artery revascularization, n (%)	171 (14%)	128 (12%)	43 (26%)	<0.0001
Sinus rhythm[a], n (%)	1209 (97%)	1055 (97%)	154 (93%)	0.0039
hs-CRP[a], mg/L	3.02 (1.34–6.76)	2.97 (1.31–6.46)	3.46 (1.53–9.43)	0.0119
sTNFR1[a], pg/mL	1786 (1532–2181)	1756 (1517–2135)	2050 (1681–2540)	<0.0001
NT-proBNP[a], pg/mL	92 (44–218)	78 (40–179)	261 (134–450)	<0.0001
S100A12, ng/mL	54 (26–150)	53 (26–139)	59 (30–223)	0.0752

ACE, angiotensin-converting enzyme; ACR, albumin-to-creatinine ratio; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; HHF, hospitalization for heart failure; hs-CRP, high sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; NT-proBNP, N terminal pro brain natriuretic peptide.
[a]Missing data: systolic/diastolic blood pressure: 7; diabetes duration: 1; HbA1c: 1; ACR: 3; smoking status: 15; sinus rhythm: 6; hs-CRP: 76; sTNFR1: 3; NT-proBNP: 5.

Figure 3 Hazard ratio (HR) for hospitalization for heart failure during the follow-up. HR are given for 1 $\log_{10}$ of S100A12 concentration. Multivariate model was adjusted for established risk factors (age, sex, duration of diabetes, history of cardiovascular disease, estimated glomerular filtration rate and urine albumin-to-creatinine ratio. The lower part of the figure shows HR according to the presence/absence of baseline chronic heart failure (CHF): N terminal pro brain natriuretic peptide concentration threshold of >1000 pg/mL was used as a surrogate to identify patients with high likelihood of pre-existing CHF.

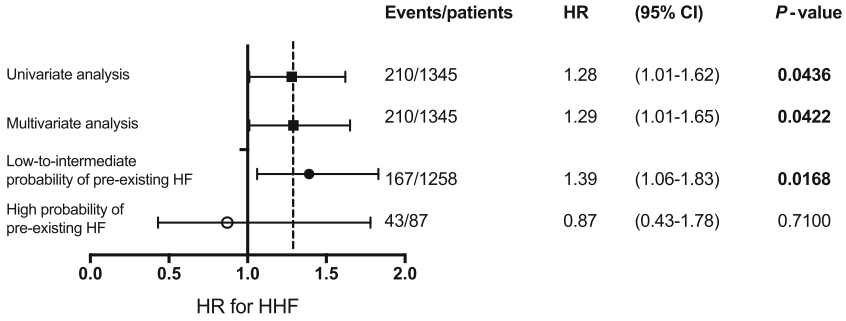


Table 4 Cox multivariate analysis for the risk of HHF in patients with NT-proBNP ≤1000 pg/mL

	HR	95% CI	P value
Age (per year)	1.06	(1.04–1.09)	<0.0001
Sex (ref. M)	1.02	(0.75–1.40)	0.8786
Duration of diabetes (per year)	1.03	(1.02–1.05)	<0.0001
eGFR (per 10 mL/min^1/1.73 m^2)	1.01	(0.92–1.11)	0.8079
ACR (per $\log_{10}$)	1.74	(1.43–2.13)	<0.0001
History of coronary heart disease	2.62	(1.92–3.60)	<0.0001
S100A12 (per $\log_{10}$)	1.39	(1.06–1.83)	0.0168

ACR, albumin-to-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-proBNP, N terminal pro brain natriuretic peptide.

prognostic value for HHF when adjusted on NT-proBNP, which remains the gold standard biomarker in all HF settings. The optimal cut-off value of S100A12 to predict HHF was >49 ng/mL; above this cut-off point, patients had an increased risk of HHF of 63%. However, the addition of S100A12 categories on top of the multivariate model enriched by NT-pro BNP did not improve the ability of the model to predict HHF. Altogether, these data suggest that the potential clinical interest of S100A12 for the prediction of HF events is limited. Nevertheless, S100A12 is an interesting marker in the specific context of diabetes and could be a

potential candidate for a future multimarker approach for CV risk assessment in diabetic patients.

S100A12 is a particularly interesting member of the large S100-protein family, showing a strong potential to activate the receptor RAGE in the context of diabetes.[29] Indeed, the pro-inflammatory properties of S100A12 are likely stable in oxidative environments such as the chronic low-grade inflammation processes observed in diabetes setting.[30] Accordingly, an association between higher circulating levels of S100A12 and incident pre-diabetes and type 2 diabetes was reported.[31] In addition, a recent study revealed differences in 27 of 74 biomarkers of inflammation (including S100A12), between several diabetes subgroups in recent adult-onset diabetes.[23] To the best of our knowledge, only two prospective studies focused on the association between circulating S100A12 concentrations and the risk of CV events in patients with type 2 diabetes: The first found an association between elevated serum S100A12 and the risk for severe peripheral arterial disease or death.[21] In the second study, combining six prospective community cohorts with diabetes from Sweden, four new biomarkers including S100A12 were identified for the risk of MACE.[22] The combined measurement of these four inflammatory proteins improved the prediction of myocardial infarction and stroke in patients with type 2 diabetes.[22] In contrast, in a recent prospective study of seniors in Sweden,[32] including only 11–14% type 2 diabetes subjects, a proteomic analysis revealed that S100A12 was only the 21th protein associated with HF; this suggests that S100A12 is a more specific marker in the hyperglycaemic pro-inflammatory environment of diabetes. The fact that S100A12 is predominantly expressed in neutrophils contributing to innate immune responses, and that it is a strong and stable RAGE ligand in the context of hyperglycaemia and diabetes, could confer a specific value to this protein as an inflammatory marker.

There might be two additional values of the present work: First, to the best of our knowledge, this is the first clinical study putting forward the S100A12 pathway specifically in the context of HF and diabetes, and these preliminary findings might stimulate further research in this field. Second, if the association of circulating S100A12 with the risk of HF was confirmed by larger cohorts, this would promote S100A12 as a candidate for a multimarker approach for more precise risk prediction than provided by NT-proBNP alone. We believe that specifically in the setting of diabetes, HF risk prediction could be improved in the future by the use of specific markers of fibrosis and inflammation, on top of natriuretic peptides. Novel biomarkers predictive of HF are emerging, such as the markers of tissue fibrosis and inflammation galectin-3 and soluble suppression of tumorigenicity (sST2),[33] or the soluble urokinase-type plasminogen activator receptor (suPAR), a non-specific marker of inflammation.[34] These biomarkers appear to reflect global inflammation and cardiac fibrosis, while S100A12 could be preferentially predictive of HHF in the context of diabetes. Combination of these novel biomarkers with S100A12 might be helpful to improve CV risk stratification in diabetes. Serum concentrations of these novel biomarkers are however not yet available in the SURDIAGENE cohort.

S100A12 predicts heart failure in patients with type 2 diabetes

The pathophysiological mechanisms linking S100A12 to HF have only been characterized in animal models. Because S100A12 is not expressed in mice, transgenic mouse models were engineered to express human S100A12, S100A8, and S100A9.[7,18] In transgenic mice with high serum S100A12 concentrations, expression of genes associated with inflammation or neutrophils activation was increased.[18] In addition, in the context of chronic kidney disease, S100A12 overexpression in mice was associated with a pathological cardiac remodelling characterized by left ventricular hypertrophy and diastolic dysfunction,[18] key mechanisms in HF. Furthermore, these dysfunctions were associated with aortic valve sclerosis and ectopic cardiac calcification, as a result of fibroblast growth factor 23 produced by cardiac fibroblasts in response to the inflammatory milieu mediated by S100A12.[7,18] These animal studies therefore strongly suggest a direct involvement of S100A12 in the pathogenesis of HF.

Data from clinical studies are less clear. In the Rotterdam study conducted in subjects without CAD or HF, elevated S100A12 concentrations were associated with a higher risk of incident CAD, in particular myocardial infarction.[17] In patients with established chronic HF, an association between S100A12 and NT-proBNP concentrations and echo-cardiac parameters (with a negative correlation with left ventricular ejection fraction) was reported.[12] Very few studies reported an association between S100A12 and HF occurrence, and none focused on patients with diabetes.

A first study, performed in Japanese patients with stable CAD, of which 43% had diabetes, showed that high S100A12 concentrations predicted the development of HF within <3 years.[35] In this study, high concentrations of S100A12 correlated with inflammation markers and a lower LEVF.[35] To explain the role of S100A12 in HF, the authors speculated that S100A12 was associated with impairment of the cardiac microcirculation and endothelial dysfunction in patients with CAD.[35] Of note, the number of HF events in this particular study was small (29 out of 652 patients developed HF) when compared with our study (210 HHF in 1345 patients).

In patients with acute coronary syndrome, of which 24% had diabetes, high concentrations of S100A12 measured 24 h after acute coronary syndrome correlated with a higher risk for HF-re-hospitalizations.[36] Finally, in patients with Stage-2 chronic kidney disease, of which 21% had diabetes,

left ventricular dysfunction was correlated to increased S100A12 concentrations.[37]

Taken together, these few data suggest that increased serum S100A12 might be associated with cardiac dysfunction, beyond its role in low-grade inflammation. As far as we know, our data are the first to suggest that high concentrations of circulating S100A12 are associated with a modest, but significant, increased risk of HHF in diabetic patients. On top of NT-proBNP, S100A12 did not have an additional prognostic value for HF prediction in this cohort. Indeed, over the past two decades, no HF biomarker performed better than NT-proBNP in all HF settings, and it is still the gold standard, even if in some specific settings other biomarkers such as galectin-3 or sST2 have been proven promising. If our results are confirmed, NT-proBNP and S100A12 might complement each other in the risk prediction of HF, the former being adapted in virtually all HF settings and the latter being more specific to the hyperglycaemic pro-inflammatory environment.

S100A12 does not predict MACE in type 2 diabetes patients

Experimental studies performed in transgenic mice for S100A12 strongly suggest that S100A12 promotes atherosclerosis, plaque vulnerability, aortic aneurysm, and vascular calcifications (for a review, please refer to Bowman and Schmidt[38]). Conversely, pharmacological inhibition of S100A12[39] reduces atherosclerosis and vascular calcification.[40] In humans, circulating concentrations of S100A12 improved the Framingham score in a prospective population-based study,[17] are higher in CAD patients when compared with healthy controls,[9–11] and are associated with progression of vascular calcification score or atherosclerosis in haemodialysis patients.[13–15] A strong association between serum S100A12 and atherosclerotic diseases,[13,41,42] including carotid intima-media-thickness,[15] peripheral artery disease,[43] and CAD,[10,14,44] was found. Taken together, there is strong evidence that high S100A12 is associated with increased long-term incidence of arterial events,[5,35,36] independently of the presence of diabetes.[12,13,17]

In diabetes populations that are at particularly high risk for CV complications, there is still limited information regarding the relationship between S100A12 and CAD. S100A12 circulating concentrations predict MACE and/or death in various prospective studies and populations, but these studies are not focused on diabetes. In patients with diabetes, a multiplex proteomics approach identified four novel biomarkers, including S100A12, for prediction of MACE; high expression of these four markers improved the risk prediction of myocardial infarction and stroke.[22] Of note, because of the methods used in the latter study, concentration values of serum S100A12 were not available, and comparisons with our study are difficult.

Surprisingly, in our cohort of type 2 diabetes patients, S100A12 concentrations did not predict MACE. However, due to heterogeneities in the study population (primary vs. secondary prevention, proportion of diabetics, mean age) and in the definition of MACE ['nonfatal stroke, nonfatal myocardial infarction, and CV death (classical 3-point MACE)', or 'CV events, admission for HF, ischemic CV events, cardiac death', or 'CV death, HHF and myocardial infarction'], head-to-head comparisons between cohorts have limited value, and apparent discrepancies can be reconciled. Another possible explanation is that hyperglycaemia and AGEs/RAGE molecular pathways may not be the major determinant of diabetic macrovascular inflammatory disease, as suggested by others (for a review, please refer to Brownlee[45]). In the particular highly macrovascular inflammatory environment of diabetes, S100A12 may not play a key role and does not seem to significantly contribute to MACE, in contrast to HHF. This remains to be demonstrated.

Limitations of the study

First, cardiac imaging was not required for inclusion in the SURDIAGENE cohort, and therefore, the presence of chronic HF was not systematically assessed. The SURDIAGENE cohort was planned in the early 2000s focusing on classical MACE and renal endpoints, and not on HF at that time.[25] Thus, history of CAD, stroke, peripheral artery disease, and renal function were well documented at inclusion, whereas HF was not. The best surrogate that we found to identify *post-hoc* patients with probable pre-existing HF was NT-proBNP that was available in the entire cohort. For the sensitivity analyses focusing on patients with low-to-intermediate likelihood of pre-existing CHF, we defined three subgroups of patients with low ($<$125 pg/mL), intermediate (125–1000 pg/mL), and high ($>$1000 pg/mL) likelihood of CHF at inclusion. The 1000 pg/mL threshold, arbitrary and by definition imperfect, was based on inclusion criteria used in recent large HF studies.[26,27] Second, although S100A12 predicted a higher risk of HHF, S100A12 concentrations were not significantly higher (P = 0.10) between patients with incident HF and patients that remained HF-free. This is in not in favour to promote S100A12 as an independent biomarker for HF.

Third, the risk prediction of HF remains modest, and on top of NT-pro-BNP, S100A12 did not have an additional prognostic value, limiting its potential interest in clinical practice.

Finally, this study includes only one biomarker, measured once at baseline and not during the follow-up.

Conclusions

In patients with type 2 diabetes, baseline serum S100A12 concentrations were independently associated with a nearby

40% increased risk of HHF events, but not with MACE. S100A12 has no incremental value for HF prediction on top of the gold standard NT-proBNP in this setting. In the particular setting of diabetes, HF risk prediction is likely to be improved in the future by the use of specific markers of fibrosis and inflammation, on top of natriuretic peptides.

This is the first clinical study putting forward the S100A12 pathway specifically in the context of HF and diabetes. If the association of serum S100A12 with the risk of incident HF was confirmed by larger cohorts, this would promote S100A12 as a candidate for a multimarker approach for more precise HF risk prediction than provided by NT-proBNP alone.

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Conflict of interest

None declared.

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Baseline data case review

All participant records were reviewed to ascertain the following points: type 2 diabetes, diabetic kidney disease, diabetic retinopathy, and cardiovascular disease.

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