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ORIGINAL RESEARCH ARTICLE

Impact of diabetes on serum biomarkers in heart failure with preserved ejection fraction: insights from the TOPCAT trial

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Abstract

Aims Diabetes mellitus (DM) is common in heart failure with preserved ejection fraction (HFpEF). Patients with DM and heart failure with reduced ejection fraction have higher levels of cardiac, profibrotic, and proinflammatory biomarkers relative to non-diabetics. Limited data are available regarding the biomarker profiles of HFpEF patients with diabetes (DM) vs. no diabetes (non-DM) and the impact of spironolactone on these biomarkers. This study aims to address such gaps in the literature.

Methods and results Biomarkers were measured at randomization and at 12 months in 248 patients enrolled in Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist's North American cohort. At baseline, DM patients had significantly lower estimated glomerular filtration rate and higher high-sensitivity C-reactive protein, pro-collagen type III amino-terminal peptide, tissue inhibitor of metalloproteinase 1 (TIMP-1), and galectin-3 levels than those without diabetes. There was a significantly larger 12 month increase in levels of high-sensitivity troponin T (hs-TnT), a marker of myocyte death, in DM patients. Elevated pro-collagen type III amino-terminal peptide and galectin-3 levels were associated with an increased risk of the primary outcome (cardiovascular mortality, aborted cardiac arrest, or heart failure hospitalization) in DM patients, but not in those without diabetes. A statistically significant interaction between spironolactone and diabetes status was observed for hs-TnT and for TIMP-1, with greater biomarker reductions among those with diabetes treated with spironolactone.

Conclusions The presence of diabetes is associated with higher levels of cardiac, profibrotic, and proinflammatory biomarkers in HFpEF. Spironolactone appears to alter the determinants of extracellular matrix remodelling in an anti-fibrotic fashion in patients with diabetes, reflected by changes in hs-TnT and TIMP-1 levels over time.

Keywords Heart failure; Preserved left ventricular function; Diabetes; Biomarker; Spironolactone

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Introduction

Half of patients with heart failure (HF) have a preserved left ventricular (LV) ejection fraction (HFpEF).[1] The prevalence of HFpEF relative to heart failure with reduced ejection fraction (HFrEF) continues to rise and, as such, has become a growing health concern.[2,3] Diabetes mellitus (DM) is a common co-morbid condition in HF, shown to be more prevalent in patients with HFpEF than in those with HFrEF.[2,4] The clinical outcomes associated with HF are considerably worse for patients with DM.[5]

Diabetic cardiomyopathy was described as its own entity by Rubler *et al.* in 1972.[6] The term is now used to refer to ventricular dysfunction in diabetic patients that is out of proportion to the underlying vascular disease.[7] The pathophysiological mechanisms of diabetic cardiomyopathy stem

from hyperglycaemia, insulin resistance, and hyperinsulinaemia and ultimately culminate in an increase in LV myocardial diastolic stiffness, hypertrophy, and fibrosis with resultant systolic and diastolic dysfunction.[7–9]

Multiple studies[10,11] have investigated the prognostic implications of biomarker profiles in diabetic patients with HFrEF and have demonstrated that certain biomarkers, notably high-sensitivity troponin T (hs-TnT) and soluble ST2 (sST2), were independently associated with both all-cause and cardiovascular (CV) mortality.[12] Indeed, HFrEF patients with diabetes were shown to have different levels of biomarkers across a spectrum of pathophysiological domains including inflammation, cardiomyocyte stretch, angiogenesis, and renal function when compared with patients without diabetes.[9]

Limited data exist on this topic for patients with HFpEF, although it has been shown that in HFpEF, patients with DM have more signs of congestion, higher N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, and a poorer prognosis.[13] Few studies, if any, have specifically analysed the changes in biomarkers over time in HFpEF patients with vs. without DM, nor examined whether any biomarker changes occurred in response to HF treatments between groups, nor explored the prognostic implications of such biomarker differences between those with and without DM.

Using plasma samples from subjects enrolled in the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial, this study seeks to examine the baseline biomarker differences between diabetic and non-diabetic patients with HFpEF to track biomarker levels' evolution over time and to assess whether biomarker changes in response to spironolactone are different depending on whether the subjects have diabetes or not. This study also examines the relationship between biomarker levels at baseline and the primary outcome of TOPCAT.

Methods

Study design and population

The TOPCAT trial was an international multicentre, randomized, double-blind, placebo-controlled trial designed to determine whether treatment with spironolactone would reduce morbidity and mortality in patients with HFpEF compared with placebo. The study design and results have been reported.[14,15] Patients eligible for inclusion in the study were ≥50 years old with symptomatic HF and LV ejection fraction ≥45%, had controlled blood pressure, and had either a HF hospitalization in the prior 12 months or elevated natriuretic peptide levels at enrolment. Either an institutional review board or an ethics committee at each site approved the study, and all patients provided informed written consent.

At selected sites in the USA, Canada, and Russia, patients were invited to contribute samples of serum, plasma, and urine to a biorepository. Patients who agreed to participate in this sub-study provided a separate, informed written consent to have blood and urine sample collected at baseline and at the time of the 12 month study visit.

Given previously reported regional differences in baseline characteristics, study outcomes, response to spironolactone, and concentrations of spironolactone metabolites,[16–18] this analysis was focused only on the group of patients in the Americas cohort. The latter cohort of the TOPCAT trial included 1767 subjects, of which 248 underwent baseline measurements of a variety of serum biomarkers. The presence of diabetes was ascertained through an electronic case report form and was based on patient-reported history of diabetes and use of antihyperglycaemic agents and insulin.

Outcome measures

This analysis used the same primary outcome as the original TOPCAT trial, which was a composite of death from a CV cause, aborted cardiac arrest, or HF hospitalization. An independent clinical endpoints committee blinded to study drug assignment adjudicated all study outcomes for the main trial.

Statistical analysis

The subgroup of patients who participated in the biomarker study was divided into non-diabetics (non-DM) and diabetics (DM).

Baseline characteristics for each group were summarized using means and standard deviations or median (interquartile range) for continuous variables and using counts and percentages for categorical variables. Characteristics were compared between groups using trend tests (linear regression, Cuzick's non-parametric trend test, and χ^2 test for trend, respectively). Per cent changes in biomarker levels over 12 months and in response to spironolactone vs. placebo were compared via linear regression after log transformation and adjustment for age, gender, strata, and treatment group. Associations between biomarkers and clinical outcomes were analysed via Cox proportion hazards model after log transformation and standardization such that hazard ratios (HRs) are comparable across biomarkers, adjusted for the same covariates described earlier (age, gender, strata, treatment, and baseline biomarker values). Effect modification between each biomarker and diabetic status and/or randomized treatment group with respect to clinical outcomes was assessed via the introduction of interaction terms to the Cox models (adjustment for treatment–biomarker and DM–biomarker interactions). Of note, DM–treatment interaction terms were not included given the insufficient number of events to detect subtle differences, with any resultant nominally significant *P*-values likely representing false-positive results. All

analyses were conducted using STATA 14.2 (College Station, TX). *P*-values <0.05 were considered statistically significant. No adjustment was made for multiple comparisons.

Results

Of the 1767 TOPCAT subjects enrolled in the Americas cohort, 248 (14.0%) had available baseline biomarkers. Compared with patients who were not included in the biomarker cohort, included subjects were less likely to be black, had lower systolic blood pressure and heart rate, had lower prevalence of hypertension and atrial fibrillation, and were more likely to be enrolled from the natriuretic peptide stratum, although their overall characteristics were otherwise similar. Of these patients for whom baseline biomarkers were available, 132 (53.2%) were non-diabetic and 116 (46.8%) had DM.

Baseline patient characteristics

Baseline characteristics according to diabetes status are summarized in *Table 1*. Diabetic patients tended to be younger and were more likely to have been identified for enrolment via the hospitalization stratum. Further, diabetic patients had a significantly higher body mass index and more often had prior myocardial infarction, percutaneous coronary interventions, peripheral artery disease, and dyslipidaemia. Atrial fibrillation was more frequent in patients without DM than in those with DM. Patients with DM had lower estimated glomerular filtration rate (eGFR) and higher blood urea nitrogen levels.

Baseline biomarker differences

The baseline biomarker differences between non-DM and DM patients with HFpEF are summarized in *Table 2*. DM patients had significantly worse renal function, as demonstrated by lower eGFR values, higher urinary protein to creatinine ratio, and higher urinary protein levels. The inflammatory biomarker high-sensitivity C-reactive protein was higher in patients with diabetes. Similar findings were observed for the inflammation-related biomarker uric acid. The following differences were observed for the myocardial extracellular matrix and myocardial fibrosis-related biomarkers: pro-collagen type III amino-terminal peptide (PIIINP), tissue inhibitor of metalloproteinase 1 (TIMP-1), and galectin-3 (Gal-3) were significantly higher in diabetic patients, while for soluble ST2 (sST2), pro-collagen type I carboxy-terminal peptide (PICP), collagen type I (CITP), matrix metalloproteinase 2 (MMP-2), and matrix metalloproteinase 9 (MMP-9), no statistically significant differences were observed according to diabetes status. There were no differences between groups at baseline in levels of

Table 1 Baseline characteristics divided by diabetes status in patients with heart failure with preserved ejection fraction

	Non-DM (n = 132)	DM (n = 116)	P for trend
Age (years)	74.3 ± 9.7	69.0 ± 9.3	<0.001
Female	67 (50.8%)	46 (39.7%)	0.08
Race			0.06
White	118 (89.4%)	92 (79.3%)	
Black	10 (7.6%)	20 (17.2%)	
Other	4 (3.0%)	4 (3.4%)	
Hispanic	2 (1.5%)	5 (4.3%)	0.18
Canada	35 (26.5%)	24 (20.7%)	0.28
BMI	31.6 ± 6.5	37.1 ± 7.7	<0.001
Strata: hospitalization	44 (33.3%)	69 (59.5%)	<0.001
Medical history			
CVD	65 (49.2%)	72 (62.1%)	0.043
MI	20 (15.2%)	33 (28.4%)	0.011
HTN	120 (90.9%)	112 (96.6%)	0.07
Stroke	10 (7.6%)	9 (7.8%)	0.96
CABG	28 (21.2%)	34 (29.3%)	0.14
PCI	23 (17.4%)	37 (31.9%)	0.008
Angina	39 (29.5%)	45 (38.8%)	0.12
COPD	14 (10.6%)	14 (12.1%)	0.72
Asthma	14 (10.6%)	17 (14.7%)	0.34
PAD	10 (7.6%)	19 (16.4%)	0.031
Dyslipidaemia	96 (72.7%)	98 (84.5%)	0.025
ICD	7 (5.3%)	4 (3.4%)	0.48
Pacemaker	17 (12.9%)	12 (10.3%)	0.54
AF	79 (59.8%)	44 (37.9%)	<0.001
Smoking status			0.22
Current	8 (6.1%)	6 (5.2%)	
Former	71 (53.8%)	75 (64.7%)	
Never	53 (40.2%)	35 (30.2%)	
NYHA			0.17
1	7 (5.3%)	3 (2.6%)	
2	73 (55.3%)	69 (59.5%)	
3	52 (39.4%)	41 (35.3%)	
4	0 (0.0%)	3 (2.6%)	
Heart rate (b.p.m.)	68.5 ± 11.3	67.4 ± 10.7	0.42
SBP (mmHg)	123.1 ± 13.8	126.6 ± 14.6	0.06
EF (%)	58.8 ± 7.2	57.4 ± 7.8	0.15
eGFR (mL/min/1.73 m^2)	67.3 ± 18.7	60.6 ± 20.2	0.007
Potassium (mmol/L)	4.1 ± 0.4	4.3 ± 0.4	<0.001
Sodium (mmol/L)	139.4 ± 2.8	139.4 ± 3.5	0.83
BUN (mmol/L)	23.0 ± 10.2	28.5 ± 14.7	<0.001
Medications			
ACE	58 (43.9%)	59 (50.9%)	0.28
ARB	38 (28.8%)	39 (33.6%)	0.41
Beta-blocker	106 (80.3%)	102 (87.9%)	0.1
CCB	49 (37.1%)	49 (42.2%)	0.41
Diuretics	115 (87.1%)	109 (94.0%)	0.07
Aspirin	67 (50.8%)	84 (72.4%)	<0.001
Nitrate	22 (16.7%)	30 (25.9%)	0.08
Statin	86 (65.2%)	99 (85.3%)	<0.001
Warfarin	68 (51.5%)	33 (28.4%)	<0.001

ACE, angiotensin-converting enzyme; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI, body mass index; BUN, blood urea nitrogen; CABG, coronary artery bypass grafting; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DM, diabetes mellitus; EF, ejection fraction; eGFR, estimated glomerular filtration rate; HTN, hypertension; ICD, implantable cardioverter-defibrillator; MI, myocardial infarction; NYHA, New York Heart Association; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; SBP, systolic blood pressure.

Table 2 Baseline biomarker differences between patients with heart failure with preserved ejection fraction without and with diabetes

Biomarker	*n*	Non-DM (*n* = 132)	DM (*n* = 116)	*P* for trend
eGFR (mL/min/1.73 m^2)	248	67 (57, 77)	57 (46, 73)	0.003
UPCR (mg/mmol)	240	0.10 (0.07, 0.15)	0.13 (0.08, 0.29)	0.001
Urinary protein level (mg/dL)	240	7.0 (4.2, 13.0)	9.8 (4.8, 23.6)	0.005
hs-CRP (mg/L)	232	2.4 (1.1, 5.6)	3.1 (1.6, 7.5)	0.046
Uric acid (mg/dL)	236	6.9 (5.6, 8.4)	7.5 (6.4, 9.2)	0.009
NT-proBNP (pg/mL)	237	624 (338, 1235)	629 (278, 1429)	0.80
hs-TnT (ng/mL)	237	5.7 (3.1, 12.4)	7.1 (3.7, 14.2)	0.17
sST2 (ng/mL)	235	28 (22, 32)	28 (21, 35)	0.36
Aldosterone (ng/L)	242	149 (120, 202)	142 (113, 174)	0.09
PICP (ng/mL)	218	140 (107, 169)	127 (102, 155)	0.29
CITP (ng/mL)	152	2.1 (1.1, 3.6)	1.6 (0.9, 3.0)	0.93
PIIINP (ng/mL)	218	22 (16, 30)	28 (21, 36)	<0.001
MMP-2 (ng/mL)	245	390 (313, 449)	411 (353, 463)	0.09
MMP-9 (ng/mL)	245	312 (212, 479)	335 (258, 474)	0.12
TIMP-1 (ng/mL)	245	188 (170, 212)	212 (183, 245)	<0.001
Gal-3 (ng/mL)	236	20 (16, 23)	22 (18, 28)	<0.001

CITP, collagen type I; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; Gal-3, galectin-3; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, pro-collagen type I carboxy-terminal peptide; PIIINP, pro-collagen type III amino-terminal peptide; sST2, soluble ST2; TIMP1, tissue inhibitor of metalloproteinase 1; UPCR, urinary protein to creatinine ratio.

the biomarker of myocardial stretch or wall stress, NT-proBNP, nor in levels of the biomarker of myocyte death, hs-TnT.

Biomarker differences over time

Of the 248 patients who provided baseline biomarkers, 204 (82.3%) provided 12 month biomarkers as well. The changes in biomarker levels of non-DM and DM patients can be found in *Table 3*. After multivariate adjustment, changes in levels of all biomarkers were comparable over time in those with and without diabetes, with the exception of hs-TnT, which showed virtually no change in non-DM patients [−1% (−14%, +13%)] but increased in DM patients [+11% (−3%, +27%)], adjusted *P* = 0.016.

Table 3 Biomarker differences over a 12 month period between patients with heart failure with preserved ejection fraction without and with diabetes

		% change (95% CI)[a]		Trend	
Biomarker	*n*	Non-DM	DM	*P*[b]	Adj[c] *P*
eGFR (mL/min/1.73 m^2)	225	−14% (−17, −10)	−14% (−18, −10)	0.38	0.34
UPCR (mg/mmol)	187	−2% (−14, +10)	−6%, (−20, +11)	0.20	0.10
Urinary protein level (mg/dL)	187	−4% (−19, +12)	−2% (−20, +20)	0.20	0.24
hs-CRP (mg/L)	183	+1% (−5, +3)	−11% (−27, +7)	0.74	0.54
Uric acid (mg/dL)	188	−1% (−5, +3)	+0% (−5, +6)	0.12	0.23
NT-proBNP (pg/mL)	189	−2% (−12, +10)	−5% (−21, +13)	0.90	0.48
hs-TnT (ng/mL)	189	−1% (−14, +13)	+11% (−3, +27)	0.06	0.016
sST2 (ng/mL)	188	−1% (−7, +5)	−4% (−9, +2)	0.54	0.60
Aldosterone (ng/L)	200	+17% (+9, +26)	+23% (+14, +33)	0.58	0.76
PICP (ng/mL)	164	+6% (−5, +18)	+5% (−6, +18)	0.22	0.10
CITP (ng/mL)	78	−25% (−45, +2)	−19% (−38, +6)	0.77	0.89
PIIINP (ng/mL)	168	+8% (−1, +18)	+10% (+1, +20)	0.11	0.15
MMP-2 (ng/mL)	203	−0% (−4, +4)	−3% (−7, +1)	0.59	0.72
MMP-9 (ng/mL)	203	−6% (−13, +3)	−1% (−11, +10)	0.23	0.35
TIMP-1 (ng/mL)	203	−1% (−4, +2)	−2% (−5, +2)	0.67	0.59
Gal-3 (ng/mL)	189	+6% (+3, +10)	+9% (+4, +14)	0.27	0.52

CI, confidence interval; CITP, collagen type I; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; Gal-3, galectin-3; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, pro-collagen type I carboxy-terminal peptide; PIIINP, pro-collagen type III amino-terminal peptide; sST2, soluble ST2; TIMP-1, tissue inhibitor of metalloproteinase 1; UPCR, urinary protein to creatinine ratio.
[a]Change in geometric means estimated via linear regression after log transformation.
[b]Adjusted for baseline value of biomarker only.
[c]Additionally adjusted for age, gender, strata, and treatment group (spironolactone vs. placebo per Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist randomization).

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Effect of spironolactone on biomarker differences between groups over time

The per cent changes reflecting treatment effect on biomarkers over time among non-DM and DM patients are compared in *Table 4* (and additionally expanded on in Supporting Information, *Table S1*). Spironolactone was associated with numerical decreases over a 12 month period in eGFR, urinary protein to creatinine ratio, urinary protein level, high-sensitivity C-reactive protein, NT-proBNP, sST2, PICP, and MMP-2 for both DM and non-DM, but these changes were not statistically significant. However, the difference in response to spironolactone observed in non-DM vs. all DM patients was statistically significant for hs-TnT [+9% (−13%, +37%) vs. −28% (−44%, −7%), interaction P = 0.027] and for TIMP-1 [+2% (−4%, +9%) vs. −8% (−14%, −2%), interaction P = 0.024].

It is important here to consider that *Table 4* provides information about the 'effect' of spironolactone, referring to the between-group difference. Specifically, the spironolactone-induced between-group difference for hs-TnT can be broken down as follows and is remarkably different between patients with and without diabetes:

$$\text{Non} - \text{DM, placebo}: N = 55, \text{change} = -1.6\%;$$

$$\text{Non} - \text{DM, spironolactone}: N = 48, \text{change} = -0.6\%;$$

$$\text{DM, placebo}: N = 42, \text{change} = +30.7\%;$$

$$\text{DM, spironolactone}: N = 44, \text{change} = -5.1\%.$$

Associations between biomarkers and clinical events

During a mean follow-up time of 2.6 ± 1.5 years, 69 (29%) patients in the biomarker cohort experienced the composite primary outcome, including 35 (15%) CV deaths and 46 (19%) HF hospitalizations, with 12 patients experiencing both outcomes. Of the 46 HF hospitalizations, 21 (46%) subjects were hospitalized once, 13 (28%) were hospitalized twice, and 12 (26%) were hospitalized more than two times. No aborted cardiac arrests were reported in the follow-up period in this group of patients.

By study group, the primary outcome event rates for the 248 patients for whom complete biomarker data were available are as follows: 27 of 132 (20%) non-DM patients and 47 of 116 (41%) DM patients. Without adjusting for biomarkers, DM patients [HR 2.21 (1.37, 3.55)] had a statistically significant increased risk of the primary outcome.

As seen in *Table 5*, when adjusted for age, sex, and randomization stratum, higher eGFR in patients with DM was associated with a lower risk of the primary outcome of TOPCAT [HR 0.56 (0.41, 0.78)]. Higher levels of PIIINP were associated with a significant increase in risk of the primary outcome among DM patients [HR 2.42 (1.62, 3.61)]. The increase in risk was not statistically significant among non-DM patients [HR 1.20 (0.80, 1.81)], and the difference in risk between groups was statistically significant (P = 0.019). Similarly, elevated Gal-3 levels were associated with a statistically significant (P = 0.034) increase in the risk of the primary outcome among DM patients with HFpEF [HR 2.12 (1.44, 3.13)] compared with non-DM patients with HFpEF [HR 1.15 (0.74, 1.81)]. After

Table 4 Effect of spironolactone vs. placebo on biomarker values in patients with heart failure with preserved ejection without and with diabetes

Biomarker	*n*	Spironolactone effect (% change)		Interaction *P*[a]
		Non-DM	DM	
eGFR (mL/min/1.73 m^2)	225	−15% (−22, −8)	−10% (−17, −2)	0.29
UPCR (mg/mmol)	187	−21% (−37, −2)	−1% (−27, +36)	0.21
Urinary protein level (mg/dL)	187	−25% (−44, −0)	−1% (−32, +45)	0.27
hs-CRP (mg/L)	183	−8% (−35, +29)	−13% (−40, +25)	0.86
Uric acid (mg/dL)	188	−1% (−8, +7)	+2% (−8, +12)	0.68
NT-proBNP (pg/mL)	189	−4% (−24, +20)	−31% (−50, −4)	0.10
hs-TnT (ng/mL)	189	+9% (−13, +37)	−28% (−44, −7)	0.027
sST2 (ng/mL)	188	−10% (−21, +1)	−16% (−25, −7)	0.34
Aldosterone (ng/L)	200	+25% (+9, +42)	+16% (+1, +34)	0.50
PICP (ng/mL)	164	−9% (−25, +11)	−23% (−36, −7)	0.24
CITP (ng/mL)	78	−8% (−44, +50)	+22% (−29, +108)	0.30
PIIINP (ng/mL)	168	+12% (−4, +30)	+6% (−11, +27)	0.77
MMP-2 (ng/mL)	203	−11% (−17, −5)	−7% (−14, +0)	0.38
MMP-9 (ng/mL)	203	+17% (−0, +38)	+9% (−9, +31)	0.74
TIMP-1 (ng/mL)	203	+2% (−4, +9)	−8% (−14, −2)	0.024
Gal-3 (ng/mL)	189	+9% (+2, +16)	+2% (−7, +11)	0.18

CITP, collagen type I; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; Gal-3, galectin-3; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, pro-collagen type I carboxy-terminal peptide; PIIINP, pro-collagen type III amino-terminal peptide; sST2, soluble ST2; TIMP-1, tissue inhibitor of metalloproteinase 1; UPCR, urinary protein to creatinine ratio.
[a]Adjusted for baseline value of biomarker.

Table 5 Hazard ratio (95% confidence interval) per standard deviation of log-transformed biomarker for primary outcome between heart failure with preserved ejection fraction patients without vs. with diabetes, adjusted for age, sex, and randomization stratum

Biomarker	Non-DM n = 132 (27 events)	DM n = 116 (47 events)	Interaction P[a]
eGFR (mL/min/1.73 m^2)	1.21 (0.71, 2.04)	0.56 (0.41, 0.78)	0.042
UPCR (mg/mmol)	0.85 (0.45, 1.63)	1.13 (0.87, 1.48)	0.40
Urinary protein level (mg/dL)	1.47 (0.90, 2.40)	1.27 (0.95, 1.70)	0.60
hs-CRP (mg/L)	1.27 (0.89, 1.83)	1.29 (0.96, 1.73)	0.87
Uric acid (mg/dL)	1.09 (0.72, 1.63)	1.65 (1.18, 2.29)	0.18
NT-proBNP (pg/mL)	1.59 (1.04, 2.45)	1.31 (0.97, 1.76)	0.66
hs-TnT (ng/mL)	2.05 (1.53, 2.76)	1.80 (1.31, 2.47)	0.86
sST2 (ng/mL)	1.48 (0.98, 2.24)	1.62 (1.20, 2.18)	0.64
Aldosterone (ng/L)	1.32 (0.92, 1.88)	1.17 (0.91, 1.50)	0.82
PICP (ng/mL)	0.90 (0.58, 1.42)	0.95 (0.69, 1.30)	0.82
CITP (ng/mL)	0.59 (0.37, 0.92)	0.69 (0.46, 1.03)	0.70
PIIINP (ng/mL)	1.20 (0.80, 1.81)	2.42 (1.62, 3.61)	0.019
MMP-2 (ng/mL)	1.08 (0.72, 1.62)	0.98 (0.72, 1.33)	0.98
MMP-9 (ng/mL)	0.81 (0.57, 1.15)	1.00 (0.73, 1.37)	0.47
TIMP-1 (ng/mL)	1.31 (0.89, 1.93)	1.09 (0.81, 1.48)	0.36
Gal-3 (ng/mL)	1.15 (0.74, 1.81)	2.12 (1.44, 3.13)	0.034

CITP, collagen type I; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; Gal-3, galectin-3; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, pro-collagen type I carboxy-terminal peptide; PIIINP, pro-collagen type III amino-terminal peptide; sST2, soluble ST2; TIMP-1, tissue inhibitor of metalloproteinase 1; UPCR, urinary protein to creatinine ratio.
[a]Interaction between biomarker and DM status, adjusted for biomarker–treatment interaction.

adjusting for eGFR, the baseline levels of both PIIINP and Gal-3 remained associated with the primary outcome of TOPCAT, and no other statistically significant difference appeared (Supporting Information, *Table S2*). A sensitivity analysis based on an outcome further incorporating non-CV deaths showed similar results (Supporting Information, *Table S3*).

Discussion

The pathophysiology of HFpEF varies across phenotypes, and profibrotic signalling has been related to the incidence,[19] severity,[20] and prognosis[21] of the disease. The presence of diabetes has known prognostic implications in HF.[5] The ultimate goal of our analysis was to elucidate the biomarker differences that distinguish diabetic patients with HFpEF from those without diabetes, thus gaining insight on the impact of diabetes in the pathophysiology of HFpEF. We found that the biomarker profiles of patients with HFpEF and DM differed from those without DM, with higher levels of inflammatory and profibrotic biomarkers in the former group. Spironolactone decreases levels of markers of myocyte damage (hs-TnT) and fibrosis (TIMP-1) to a further extent in patients with diabetes than in those without diabetes over 12 months of treatment. We also observed that the presence of diabetes appears to modify prognostic associations between baseline levels of eGFR, PIIINP, and Gal-3 and the primary outcome of TOPCAT.

Clear baseline biomarker differences of renal function were noted when comparing non-DM with DM patients, findings consistent with well-established literature documenting the association between diabetes and renal disease and confirming that insulin-treated diabetics experience more significant and progressive renal disease than their non-insulin-treated counterparts.[22]

Among the biomarkers analysed, of particular interest were the statistically significant differences in the cardiac remodelling-specific or profibrotic biomarkers PIIINP, TIMP-1, and Gal-3, with higher baseline levels found in patients with DM in comparison with those without DM. Maturation of newly synthesized collagen requires removal of the N-terminal propeptides. The concentration of these propeptides, such as PICP and PIIINP reflects collagen synthesis rate. Elevated PIIINP, correlating to increased profibrotic processes, has also previously been associated with increased severity of disease.[20] Collagen degradation and turnover is reflected by biomarkers such as TIMP-1 and collagen type I. Gal-3, which has been associated with HFpEF and fibrosis,[21] was also shown to be increased in patients with impaired glucose metabolism and increased haemoglobin A1c.[11,12] The median Gal-3 level among DM patients with HFpEF in this TOPCAT analysis was 22.0 ng/mL, which is slightly higher than the findings reported in HFrEF populations,[11] and clearly increased compared with the levels reported in healthy populations.[23]

There were no statistically significant differences in baseline levels, nor in change of biomarker levels over time, of NT-proBNP, a biomarker of myocardial stretch and an established marker of prognosis in HF, between non-DM and DM patients.

There were no differences in hs-TnT levels at baseline between non-DM and DM patients. However, hs-TnT was the

only biomarker to demonstrate a statistically significant change over 12 months, seen only in diabetic patients. While hs-TnT levels remained constant in non-DM patients, the levels increased in DM patients, even after adjusting for baseline biomarker levels, age, gender, randomization stratum, and treatment. Higher levels of hs-TnT have been associated with both all-cause and CV mortality in patients with HFrEF.[9] Moreover, it has recently been reported in the HFpEF population that higher troponin levels are independently associated with an increased risk of CV death and HF hospitalization.[24] While DM patients experience significant increases in hs-TnT over time compared with their non-DM counterparts, this is counteracted by reductions in hs-TnT with spironolactone treatment in those with DM.

Indeed, treatment with spironolactone differentially affected two of the biomarkers studied according to DM status at baseline, with significant decreases in levels of TIMP-1 and hs-TnT among the DM group while the levels of these same biomarkers were unchanged by treatment with spironolactone in the non-DM population. The spironolactone-induced decrease in TIMP-1 among diabetic patients with HFpEF is also noteworthy, given the role that TIMP-1 plays in the determinants of extracellular membrane structural and function remodelling in HFpEF.[8] TIMP-1 was the strongest predictor of all-cause mortality after age in a cohort of >5000 Icelandic patients[25] and was associated with LV hypertrophy and systolic dysfunction by echocardiogram in the Framingham Heart Study.[26] It appears that spironolactone does indeed exert a differential response in the pathophysiology of HFpEF based on patients' diabetes status, possibly conferring additional treatment benefit to those patients with diabetes compared with those without.

In this study, patients with HFpEF and diabetes had a poorer prognosis than those without diabetes. Of particular interest, however, is that the prognostic implications of the biomarkers studied varied according to diabetes status for two profibrotic biomarkers, Gal-3 and PIIINP, as well as for a marker of renal function, eGFR. The significance of these differences demonstrates that, in the case of Gal-3 and PIIINP, the association between biomarkers and the primary outcome differed according to diabetes status, with the association between higher biomarker levels and the primary outcome being stronger in diabetic patients. The importance of this association is magnified when considering that previous trials have demonstrated that higher levels of both PIIINP[27] and Gal-3[28] are associated with adverse outcomes in HFrEF. Similarly, our results demonstrate a strong negative association between eGFR and the primary outcome of TOPCAT (i.e. lower eGFR is associated with a higher risk of primary outcome); this association appears stronger in diabetic patients with HFpEF. Given the small number of events for this analysis, the conclusions drawn herein should be considered hypothesis generating.

Study limitations

Our study's main objective was not to assess the prognostic value of biomarkers in HFpEF patients with and without DM but rather to compare biomarker profiles and the impact of spironolactone on biomarkers between those two groups. For the outcome analysis, the number of events may have undermined the power to detect weaker clinical associations. Furthermore, given the number of models tested in relation to changes over time and clinical outcomes, there exists the possibility of type I error. Finally, only a proportion of TOPCAT subjects in the Americas cohort provided samples to the biorepository, and the results may not be applicable to other regions.

Conclusions

Significant baseline and 12 month biomarker differences exist between non-DM HFpEF patients and their DM counterparts, highlighting a more important ongoing profibrotic profile in those with DM. TIMP-1 and hs-TnT were reduced by spironolactone in patients with DM, suggesting favourable anti-remodelling effects in this group. The prognostic value of renal and fibrosis-related biomarkers in HFpEF appears to be even stronger in patients with DM than in those without DM. A 2.2-fold increased risk of experiencing the primary outcome in TOPCAT was observed in DM compared with non-DM patients.

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Conflict of interest

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Effect of spironolactone versus placebo initially (t_0) and at 12 months (t_{12}) on biomarker values in HFpEF non-DM patients versus HFpEF DM patients

Table S2: Hazard ratio (95% CI) per SD of log-transformed biomarker for primary outcome between HFpEF non-diabetic patients versus diabetic patients; adjusted for age, sex, randomization stratum, and eGFR

Table S3: Hazard ratio (95% CI) per SD of log-transformed biomarker for any death or HF hospitalization between non-DM patients versus all DM patients; adjusted for age, sex, and randomization stratum

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