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Circulating cardiovascular biomarkers in recurrent atrial fibrillation: data from the GISSI-Atrial Fibrillation Trial

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Abstract. Latini R, Masson S, Pirelli S, Barlera S, Pulitano G, Carbonieri E, Gulizia M, Vago T, Favero C, Zdunek D, Struck J, Staszewsky L, Maggioni AP, Franzosi MG, Disertori M on the behalf of the GISSI-AF Investigators (Istituto di Ricerche Farmacologiche "Mario Negri", Milan; Istituti Ospitalieri, Cremona; POL Madonna della Consolazione, Reggio Calabria; Ospedale Nuovo Girolimo Fracastoro, San Bonifacio; Ospedale Garibaldi-Nesima, Catania; Ospedale Luigi Sacco, Milan, Italy; Roche Diagnostics, Rotkreuz, Switzerland; B.R.A.H.M.S. AG, Henningsdorf, Germany; ANMCO Research Center, Florence; and Ospedale Santa Chiara, Trento, Italy). Circulating cardiovascular biomarkers in recurrent atrial fibrillation: data from the GISSI-Atrial Fibrillation Trial. *J Intern Med* 2011; **269**: 160–171.

Objective. We evaluated the prognostic role of circulating cardiovascular biomarkers in patients with a history of recent atrial fibrillation (AF).

Background. Predicting long-term maintenance of sinus rhythm in patients with AF is difficult.

Methods. Plasma concentrations of three specific cardiac markers [high-sensitivity troponin T (hsTnT), N-terminal probrain natriuretic peptide (NT-proBNP) and mid-regional proatrial natriuretic peptide (MR-proANP)] and three stable fragments of vasoactive peptides [mid-regional proadrenomedullin (MR-proADM), copeptin (CT-proAVP) and CT-proendothelin-1 (CT-proET-1)] were measured at baseline and after 6 and 12 months in 382 patients enrolled in the GISSI-AF study, a prospective randomized trial to determine the effect of valsartan to reduce the recurrence of AF. The association between these markers, clinical characteristics and recurrence of AF was tested by univariate and multivariate Cox models.

Results. Mean patient age was 68 ± 9 years (37.2% females). A total of 84.8% of patients had a history of hypertension. In total, 59.7% qualified for history of AF because of successful cardioversion, 11.8% because of two or more episodes of AF in the 6 months preceding randomization and 28.5% because of both. Patients in AF at 6 or 12 months (203 (53.1%) with first recurrence) had significantly higher concentrations of most biomarkers. Despite low baseline levels, higher concentrations of hsTnT {adjusted hazard ratio (HR) [95% confidence intervals (CIs) for 1 SD increment] (1.15 [1.04–1.28], $P = 0.007$), MR-proANP (1.15 [1.01–1.30], $P = 0.04$), NT-proBNP (1.24 [1.11–1.39], $P = 0.0001$) and CT-proET-1 (1.16 [1.01–1.33], $P = 0.03$) independently predicted higher risk of a first recurrence of AF. Changes over time of MR-proANP tended to predict subsequent recurrence (adjusted HR [95%CI]) (1.53 [0.98–2.37], $P = 0.06$).

Conclusion. Circulating markers of cardiomyocyte injury/strain and endothelin are related to recurrence of AF in patients in sinus rhythm with a history of recent AF.

Keywords: atrial fibrillation, natriuretic peptides, prognosis, troponin T.

Abbreviations: AF, atrial fibrillation; ARB, angiotensin receptor blocker; CT-proAVP, copeptin or C-terminal provasopressin; CT-proET-1, C-terminal proendothelin-1; hsTnT, high-sensitivity cardiac troponin T; MR-proADM, mid-regional proadrenomedullin; MR-proANP, mid-regional proatrial natriuretic peptide; NT-proBNP, N-terminal probrain natriuretic peptide.

*A complete list of the study investigators is presented in the Appendix.

§RL and SM contributed equally to the study.

Introduction

Atrial fibrillation (AF) recurs within the first year after successful cardioversion in about 50% of patients [1]. Predictors of recurrence may help in planning the frequency of planned clinical visits, in modulating therapy and in better understanding the pathophysiology of the disease. Whilst several studies have reported increased concentrations of cardiovascular markers in patients with AF [2–7], few studies have assessed the predictive roles of these markers in patients in sinus rhythm at high risk of AF [8, 9]. Given the complexity of studies in this field because of the heterogeneity of populations and the limitations in terms of sample size, it can be concluded that in general natriuretic peptides are elevated during AF and predict AF recurrence or first occurrence in patients with various comorbidities [3], hypertension [10] and heart failure [11], or those who have undergone cardiac surgery [12]. Major limitations of these studies include small sample size (<100 patients) and/or retrospective design.

GISSI-AF, a trial to determine the effect of valsartan in preventing recurrence of AF in patients in sinus rhythm [13], appeared to be a suitable setting to investigate how different biomarkers would predict recurrence of AF. Accordingly, six different cardiovascular markers were assayed in 382 patients enrolled in the GISSI-AF trial. High-sensitivity cardiac troponin T (hsTnT) and N-terminal probrain natriuretic peptide (NT-proBNP) were measured as sensitive and specific markers of cardiac injury/strain/filling pressures. Mid-regional proatrial natriuretic peptide (MR-proANP) was assayed as it is considered to best reflect atrial strain, a determinant of AF. Amongst the three vasoactive peptides, C-terminal proendothelin-1 (CT-proET-1) was measured because of the relation between endothelin and new-onset AF in heart failure [12] and because of its possible role in the pathogenesis of AF [14, 15]. Mid-regional proadrenomedullin (MR-proADM) and C-terminal provasopressin (copeptin) have been found to be related to the altered haemodynamics and volume load in heart failure [16, 17].

Patients and methods

The GISSI-AF trial was a randomized, double-blind, placebo-controlled, multicentre study that enrolled 1442 patients who were in sinus rhythm but had had either two or more documented episodes of AF in the previous 6 months or successful cardioversion for AF in the previous 2 weeks (qualifying history of AF). To be eligible for the study, patients also had to have underlying cardiovascular disease, diabetes or left atrial enlargement. The aim of the trial was to determine whether valsartan could reduce the recurrence of AF [13, 18]. In a subset of 382 patients recruited in 36 clinical centres (see Appendix), blood samples were drawn at randomization and after 6 and 12 months of follow-up. Patients remained supine for at least 15 min before blood was collected using an indwelling venous cannula. Blood was centrifuged at 4 °C within 10 min, and plasma aliquots were sent, on dry ice, to a central laboratory. Samples were stored for up to 2 years at –70 °C until required for assay.

Study visits were scheduled at weeks 2, 4, 8, 24 and 52. A routine clinical examination, including electrocardiography (ECG) and laboratory testing, was performed at each study visit. To increase the likelihood of AF detection, all patients were provided with a transtelephonic monitoring device (Cardiobios 1; Telbios, Milan, Italy). Patients were asked to activate the device, which would transmit a 30-s electrocardiogram to both the coordinating centre and the responsible physician, if symptoms occurred and at least once a week whether or not symptoms occurred. If a recurrence of AF was detected, patients were asked to come in for an office visit to confirm the findings. Each AF episode during the trial was adjudicated blindly by a central reader and verified by an ad hoc validation committee.

Determination of biomarker concentrations

The six biomarkers were assayed in a central laboratory in a blinded fashion in EDTA-anticoagulated plasma. Measurements of B-type natriuretic peptide and endothelin-1 were prespecified in the protocol [18]. Plasma concentrations of MR-proANP, MR-proADM, CT-proET-1 and copeptin were measured with chemiluminescence immunoassays (BRAHMS AG, Henningsdorf, Germany). The limits of detection of these assays were 6 pmol L^{-1} for MR-proANP, 0.08 nmol L^{-1} for MR-proADM, 0.4 pmol L^{-1} for CT-proET-1 and 1.7 pmol L^{-1} for copeptin. The sensitivity of the functional assay, defined as the concentration at which the interassay coefficient of variation (CV) was 10%, was 65 pmol L^{-1} for MR-proANP, 0.4 nmol L^{-1} for MR-proADM, 10 pmol L^{-1} for CT-proET-1 and 9 pmol L^{-1} for copeptin. The normal reference ranges for the four biomarkers have been published previously and are expressed as median [2.5th–97.5th percentiles] as follows: MR-proANP, 45 [18.4–163.9] pmol L^{-1}; MR-proADM, 0.33 [0.17–0.49] nmol L^{-1}; CT-proET-1, 44.3 [24.8–66.6]

pmol L^{-1}; copeptin, 4.2 [1.7–11.25] pmol L^{-1}. NT-proBNP and hsTnT were measured using an electrochemiluminescence immunoassay method (Elecsys 2010 analyzer; Roche Diagnostics, Rotkreuz, Switzerland), as previously described [19, 20]. The 99th percentile cut-off for hsTnT from 616 healthy reference subjects was 13.5 pg mL^{-1}, and the CV was 9% [21].

Statistical methods

Baseline characteristics of study patients by first AF recurrence were compared by chi-square test or by *t*-test. Concentrations of the biomarkers were expressed as median [quartile 1–quartile 3]. Median concentrations of the biomarkers were compared by nonparametric Wilcoxon rank-sum test or Kruskal–Wallis test when appropriate.

The association between biomarker concentrations and first recurrence of AF was evaluated over a follow-up period of 1 year. The association between baseline concentration of each biomarker and first recurrence of AF was assessed by univariate Cox proportional hazards models. Biomarkers were modelled as continuous variables (expressed as 1 SD increment) as linearity of the hazard was tested by appropriate transformation using restricted cubic splines to account for possible nonlinear relationships. Cox multivariate models were performed to evaluate the independent prognostic value of each biomarker separately; adjusting for baseline covariates emerged as statistically significant from the stepwise selection procedure ($P < 0.05$), i.e. qualifying history of AF, smoking, documented coronary artery disease and history of lone AF with atrial dilatation. The prognostic discrimination of each biomarker for first AF recurrence was evaluated by the area under the receiver operating characteristic curve (AUROC). We also investigated whether the addition of different combinations of the biomarkers improved the discrimination of the model. Estimates of the C-statistic for the Cox regression models were calculated according to the method of Pencina *et al.* [22]. Kaplan–Meier curves for the probability of first recurrence of AF were plotted for baseline concentrations of the six biomarkers according to their median cut-off value and compared by the log-rank test. Similarly, the independent prognostic value of each biomarker on all-cause and cardiovascular hospitalizations was assessed by univariate and multivariate Cox models.

Concentrations of all biomarkers in patients who were in sinus rhythm compared with those in AF at the 6-month and 12-month scheduled ECG recordings were compared by Wilcoxon rank-sum test.

Each biomarker measured at baseline, 6 and 12 months was analysed in terms of within-patient changes over time for first AF recurrence. A repeated-measures analysis of variance (anova) was performed on the biomarker concentrations transformed on a logarithmic scale.

To evaluate the prognostic value of changes in biomarker concentrations over 6 months on the risk of AF recurrence, relative changes of each marker [(6 months–baseline)/baseline] were calculated. For those patients in sinus rhythm at 6 months' follow-up, univariate and multivariate Cox models were used to assess the association between changes (median value cut-off) in the biomarkers and the first occurrence of AF within the next 6 months (6–12 months).

All probability values are two-tailed, and 95% confidence intervals (CIs) were calculated. Because of the exploratory nature of this study, adjustments for the multiplicity of statistical analyses have not been made. Statistical analyses were performed with sas software, version 9.1 (SAS Institute, Cary, NC, USA) and with the program R and the package rms (http://CRAN.R-project.org/package=rms).

Results

Baseline characteristics

Baseline characteristics of the study patients overall and by first recurrence of AF are shown in Table 1. The only statistically significant differences between patients who did and did not have a recurrence of AF were sex and qualifying history of AF: males and patients with two or more episodes of AF in the previous 6 months were more likely to have a recurrence of AF.

The characteristics of the 382 patients in the biomarker substudy were almost identical to those of the main GISSI-AF trial (data not shown, [13]). Accordingly, 53.1% (203/382) of patients had a first recurrence of AF over 1 year of follow-up in the substudy, compared to 51.7% (746/1442) in the main study. Similar consistency of outcomes was also found for the coprimary end-point, more than one recurrence of AF, and for the secondary end-points of GISSI-AF. The rate of all-cause deaths was 0.26% (1/382) in the substudy and 1.0% (15/1442) in the main study, and

Table 1 *Baseline patient characteristics according to first recurrence of AF*

Characteristics	All patients (n = 382)	Patients with ≥1 recurrence of AF (n = 203)	Patients without recurrence of AF (n = 179)	Pvalue
Females, n(%)	142 (37.2)	65 (32.0)	77 (43.0)	0.03
Age, mean ± SD (years)	68.1 ± 9.1	67.8 ± 9.2	68.4 ± 9.0	0.54
BMI, mean ± SD (kg m^{-2})	27.9 ± 4.3	27.9 ± 3.9	27.8 ± 4.7	0.84
Systolic blood pressure, mean ± SD (mmHg)	138.3 ± 16.5	137.4 ± 16.2	139.2 ± 16.8	0.29
Diastolic blood pressure, mean ± SD (mmHg)	81.2 ± 8.4	80.6 ± 8.7	81.8 ± 8.1	0.15
Estimated GFR, mean ± SD (ml min^{-1} 1.73 m^{-2})	72 ± 18	73 ± 17	71 ± 18	0.39
Inclusion criteria				
≥2 episodes of AF in previous 6 months, n(%)[a]	154 (40.9)	93 (46.3)	61 (34.7)	0.02
Cardioversion in previous 2 weeks, n(%)[a]	336 (88.0)	174 (85.7)	162 (90.5)	0.15
Heart failure, LVEF <40%, or both, n(%)	42 (11.0)	25 (12.3)	17 (9.5)	0.38
History of hypertension, n(%)	324 (84.8)	167 (82.3)	157 (87.7)	0.14
Diabetes mellitus, n(%)	50 (13.1)	28 (13.8)	22 (12.3)	0.66
History of stroke, n(%)	15 (3.9)	7 (3.5)	8 (4.5)	0.61
Peripheral artery disease, n(%)	22 (5.8)	15 (7.4)	7 (3.9)	0.15
Documented CAD, n(%)	41 (10.7)	25 (12.3)	16 (8.9)	0.29
Single AF episode with LA dilatation, n(%)	52 (13.6)	33 (16.3)	19 (10.6)	0.11
Duration of last qualifying episode of AF >7 days, n(%)	151 (39.5)	80 (39.4)	71 (39.7)	0.94
Comorbidities				
Peripheral embolism, n(%)	4 (1.1)	3 (1.5)	1 (0.5)	0.38
Renal dysfunction, n(%)	10 (2.6)	5 (2.5)	5 (2.8)	0.84
COPD, n(%)	33 (8.6)	21 (10.3)	12 (6.7)	0.21
Neoplasia, n(%)	12 (3.1)	4 (2.0)	8 (4.5)	0.16
Current smoking, n(%)	36 (9.4)	20 (9.9)	16 (8.9)	0.21
Alcohol abuse, n(%)	4 (1.1)	1 (0.5)	3 (1.7)	0.26
Electrocardiography findings at randomization				
Heart rate, mean ± SD (bpm)	62.2 ± 9.7	61.9 ± 9.8	62.6 ± 9.6	0.50
QRS > 120 ms, n(%)	43 (11.3)	23 (11.4)	20 (11.2)	0.95
LVH, n(%)	34 (8.9)	19 (9.4)	15 (8.4)	0.74
Pathological Q waves, n(%)	11 (2.9)	6 (3.0)	5 (2.8)	0.92
Concomitant cardiovascular therapies				
Amiodarone, n(%)	148 (38.7)	74 (36.5)	74 (41.3)	0.33
Sotalol, n(%)	28 (7.3)	18 (8.9)	10 (5.6)	0.22
Class I antiarrhythmics, n(%)	127 (33.3)	68 (33.5)	59 (33.0)	0.91
ACE inhibitors, n(%)	206 (53.9)	115 (56.7)	91 (50.8)	0.26
CCBs, n(%)	108 (28.3)	54 (26.6)	54 (30.2)	0.44
β-Blockers, n(%)	114 (29.8)	58 (28.6)	56 (31.3)	0.56
Digitalis, n(%)	16 (4.2)	10 (4.9)	6 (3.4)	0.44
Diuretics, n(%)	149 (39.0)	85 (41.9)	64 (35.8)	0.22
Aldosterone blockers, n(%)	20 (5.2)	8 (3.9)	12 (6.7)	0.23
Statins, n(%)	98 (25.7)	53 (26.1)	45 (25.1)	0.83

Table 1 (*Continued*)

Characteristics	All patients (n = 382)	Patients with ≥1 recurrence of AF (n = 203)	Patients without recurrence of AF (n = 179)	Pvalue
Oral anticoagulants, n(%)	233 (61.0)	126 (62.1)	107 (59.8)	0.65
Aspirin, n(%)	101 (26.4)	55 (27.1)	46 (25.7)	0.76
Randomized treatment, valsartan, n(%)	186 (48.7)	106 (52.2)	80 (44.7)	0.14

AF, atrial fibrillation; ACE, angiotensin-converting enzyme; BMI, body mass index; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; LVH, left ventricular hypertrophy.
[a]109 patients had both entry criteria.

all-cause hospitalizations occurred at a rate of 18.3% (70/382) in the substudy and 20.3% (292/1442) in the main study.

Biomarker concentrations in sinus rhythm and in AF

Baseline levels of the biomarkers are shown in Table 2. Concentrations of all biomarkers were higher at the 6- and 12-month follow-up visits in patients who were in AF, compared to those who were in sinus rhythm (Table 2).

NT-proBNP and MR-proANP significantly decreased over the 1-year follow-up, although the decrease was more marked over the first 6 months (Table 3). Whereas copeptin did not change significantly, MR-proADM and CT-proET1 slightly but significantly increased. The decrease in NT-proBNP and MR-proANP was significantly attenuated in patients with at least one recurrence of AF over 1 year (Table 3: interaction time × AF recurrence, P = 0.0005 and 0.0007, respectively).

Baseline NT-proBNP, MR-proANP and hsTnT were higher in patients older than 70 years, in those with heart failure and/or left ventricular ejection fraction <40%, and those with coronary artery disease (data not shown). Reduced renal filtration (estimated glomerular filtration rate ≤60 ml min^{-1} 1.73 m^{-2}) was associated with significantly higher concentrations of all six biomarkers (all P < 0.01). The six biomarkers significantly correlated with each other (P < 0.001), with Spearman nonparametric correlation coefficients ranging from 0.20 to 0.78 (the highest value was between NT-proBNP and MR-proANP).

When the concentrations of biomarkers were analysed by time, in days from the last qualifying cardioversion before randomization, lower concentrations of biomarkers were associated with longer time intervals from cardioversion. The most remarkable relation was observed for NT-proBNP: 1–7 days, 246 pg mL^{-1}; 7–16 days, 209 pg mL^{-1}; >16 days, 151 pg mL^{-1} (Kruskal–Wallis test, P < 0.0001). Similar relations were found for hsTnT (P = 0.04) and MR-proANP (P = 0.02), but not for the other markers.

Biohumoral predictors of recurrence of AF

Kaplan–Meier curves for probability of first recurrence of AF by median levels of baseline hsTnT started to diverge after the first 30 days of follow-up (Fig. 1; log-rank test, P = 0.04). Similar trends were also found for NT-proBNP (P = 0.08) and MR-proANP (P = 0.07), whereas no differences were observed for the other three vasoactive peptides.

For those patients who experienced a recurrence of AF (n = 203), an inverse relation was found between the baseline concentration of the two natriuretic peptides (NT-proBNP and MR-proANP), but not of other markers, and the time to develop the first AF recurrence (Fig. 2).

Baseline concentrations of the three specific cardiac biomarkers (hsTnT, NT-proBNP and MR-proANP) independently predicted recurrence of first AF, after adjustment for patient characteristics (Cox multivariate model, Table 4). Adjusted hazard ratios (HRs) [95% CI for 1 SD increment] were 1.15 [1.04–1.28] for hsTnT (P = 0.007), 1.24 [1.11–1.39] for NT-proBNP (P = 0.0001) and 1.15 [1.01–1.30] for MR-proANP (P = 0.04). CT-proET-1 was also associated with the risk of first recurrence of AF (1.16 [1.01–1.33], P = 0.03). The risk of having more than one episode over the 1-year follow-up period was not predicted by any of the six biomarkers (data not shown). ROC analysis for hsTnT, NT-proBNP and MR-proANP yielded specificities ranging from 0.49 to 0.57 and sensitivities ranging from 0.50 to 0.65, with optimal cut-off values of 7.3 pg mL^{-1}, 206 pg mL^{-1} and 165 pmol

Table 2 *Biomarker concentrations according to cardiac rhythm at baseline, 6- and 12-month follow-up electrocardiography visits*

	Baseline	6 months			12 months		
	SR	SR	AF	*P*	SR	AF	*P*
hsTnT (pg mL^{-1})	8.1 [5.5–12.6] (370)	7.2 [5.0–11.4] (280)	8.9 [6.6–12.3] (41)	0.05	7.2 [5.1–11.6] (275)	7.9 [5.3–14.2] (56)	0.22
NT-proBNP (pg mL^{-1})	191 [95–363] (382)	129 [56–240] (293)	700 [402–1205] (41)	<0.0001	117 [55–248] (286)	560 [308–1051] (57)	<0.0001
MR-proANP (pmol L^{-1})	162 [110–227] (380)	129 [93–192] (293)	246 [201–324] (41)	<0.0001	135 [91–197] (285)	242 [160–353] (57)	<0.0001
MR-proADM (nmol L^{-1})	0.64 [0.53–0.79] (373)	0.62 [0.52–0.78] (292)	0.71 [0.58–0.85] (39)	0.01	0.64 [0.53–0.83] (282)	0.70 [0.61–0.87] (57)	0.007
Copeptin (pmol L^{-1})	6.5 [4.1–11.1] (383)	6.7 [3.8–11.3] (293)	9.9 [5.6–14.5] (41)	0.02	6.8 [4.3–11.7] (285)	7.8 [6.1–13.5] (57)	0.09
CT-proET-1 (pmol L^{-1})	70 [60–86] (369)	69 [60–83] (291)	80 [68–99] (40)	0.004	71 [62–86] (282)	82 [68–100] (57)	0.001

Concentrations are expressed as median [quartile 1–quartile 3], for the number of patients indicated in parentheses. *P* values derived from Wilcoxon rank-sum test.

SR, sinus rhythm; AF, atrial fibrillation; NT-proBNP, N-terminal probrain natriuretic peptide; MR-proANP, mid-regional proatrial natriuretic peptide; MR-proADM, mid-regional proadrenomedullin; hsTnT, high-sensitivity cardiac troponin T; CT-proET-1, C-terminal proendothelin-1.

Table 3 *Time course of biomarker concentrations according to the first recurrence of AF*

	Patients with no AF recurrence (n = 143–161)			Patients with AF recurrence (n = 170–194)			Repeated anova *P* values	
Biomarker	Baseline	6 months	12 months	Baseline	6 months	12 months	Time	Time × AF recurrence
hsTnT (pg mL^{-1})	7.6 [5.2–10.6]	7.0 [4.9–9.9]	7.1 [4.9–10.7]	8.6 [5.7–13.5]	8.1 [5.4–11.8]	7.2 [5.2–12.3]	0.01	0.85
NT-proBNP (pg mL^{-1})	183 [92–325]	112 [55–206]	116 [48–240]	197 [98–429]	174 [74–435]	194 [76–470]	<0.0001	0.0005
MR-proANP (pmol L^{-1})	153 [108–219]	121 [90–168]	134 [88–196]	167 [112–229]	154 [107–226]	154 [105–242]	0.0005	0.0007
MR-proADM (nmol L^{-1})	0.64 [0.53–0.79]	0.61 [0.53–0.79]	0.63 [0.52–0.81]	0.64 [0.53–0.79]	0.64 [0.52–0.78]	0.67 [0.55–0.84]	0.001	0.81
Copeptin (pmol L^{-1})	6.5 [4.2–11.5]	6.8 [3.9–11.1]	7.1 [3.9–11.1]	6.4 [4.1–11.0]	6.8 [4.1–12.1]	7.0 [4.9–12.1]	0.07	0.66
CT-proET-1 (pmol L^{-1})	70 [60–84]	68 [60–82]	71 [62–84]	71 [60–87]	72 [60–85]	75 [63–90]	0.01	0.84

NT-proBNP, N-terminal probrain natriuretic peptide; MR-proANP, mid-regional proatrial natriuretic peptide; MR-proADM, mid-regional proadrenomedullin; hsTnT, high-sensitivity cardiac troponin T; CT-proET-1, C-terminal proendothelin-1; AF, atrial fibrillation.

The number of patients varies according to the biomarker measured and time of visit. *P* values are for repeated anova on log-transformed biomarker concentrations. At least one recurrence of AF any time over 1 year abolished the decrease in natriuretic peptide concentrations.

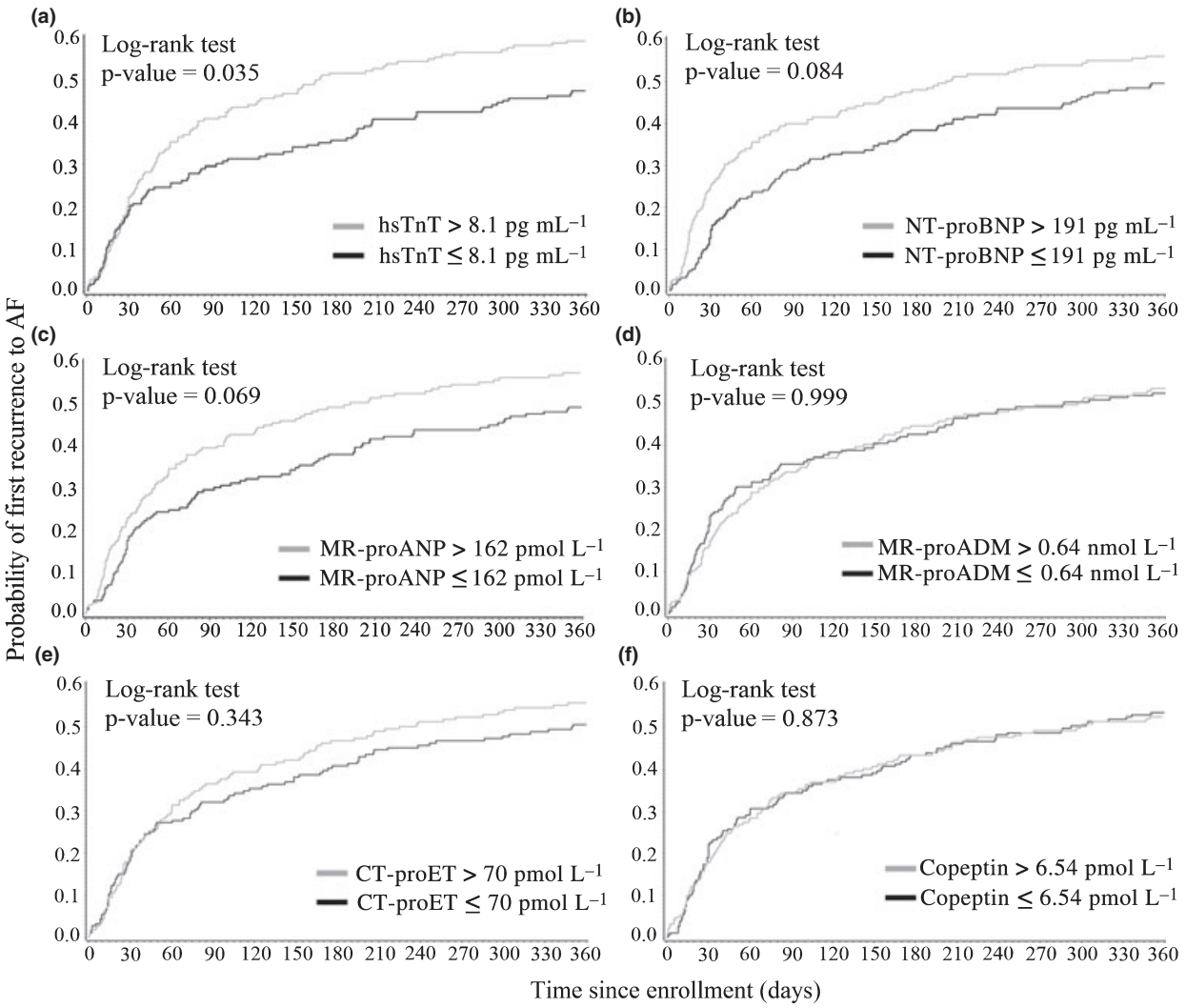

Fig. 1 *Kaplan–Meier curves for probability of first recurrence of atrial fibrillation by median of baseline concentrations of (a) hsTnT, (b) NT-proBNP, (c) MR-proANP, (d) MR-proADM, (e) CT-proET, and (f) copeptin.*

L^{-1}, respectively. Except for hsTnT ($P = 0.01$), none of the biomarkers had a C-statistic significantly different from the line of no discrimination. The C-statistic increased for the prediction of first AF recurrence when all six biomarkers were incorporated into a model with clinical risk factors and NT-proBNP (AUROC from 0.590 to 0.602, $P = 0.02$).

Patients were then divided according to relative changes of biomarker concentrations (above or below median) over the first 6 months of follow-up. Recurrence of AF within the subsequent 6 months was more frequent in patients with above the median changes in MR-proANP (141 patients with relative changes from −9% to +1380%; 60.9% of these patients had an episode of AF recurrence) than in those with below the median changes (141 patients with relative changes from −82% to −9%; 39.1% of patients with AF recurrence, $P = 0.01$), with a univariate HR of 1.77 [1.15–2.72] ($P = 0.009$). After adjustment for relevant clinical risk factors, the risk of recurrence of AF in patients with above the median 6-month changes in MR-proANP was still moderately elevated but no longer significant (HR 1.53 [0.98–2.37], $P = 0.06$). Similarly, recurrence of AF tended to be more frequent in patients with above the median changes in NT-proBNP (142 patients with relative changes from −21% to +786%; 58.1% of patients with AF

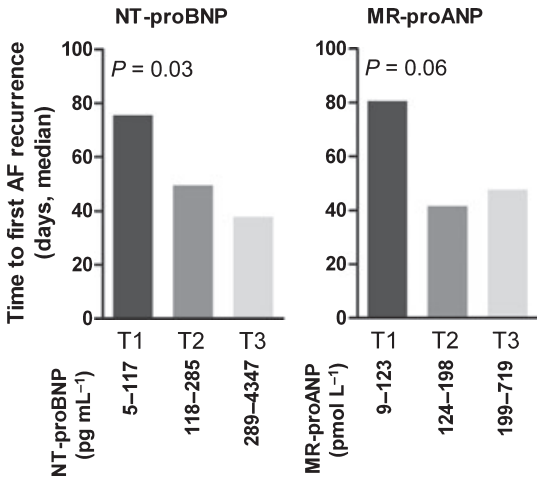

Fig. 2 *Time to first recurrence of atrial fibrillation by tertiles of baseline concentrations of the natriuretic peptides, N-terminal proBNP and mid-regional proANP.* P *value determined by Kruskal–Wallis test. T, tertile.*

recurrence) than in those with below the median changes (142 patients with relative changes from −99% to −21%; 41.9% of patients with AF recurrence, $P = 0.07$). Univariate HR was 1.57 [1.02–2.41] ($P = 0.04$) but was no longer significant after adjustment ($P = 0.29$). Relative changes over time for the other four biomarkers were not associated with subsequent AF recurrence.

Biohumoral predictors of all-cause and cardiovascular hospitalizations

One-year incidences of hospitalizations for any cause and for cardiovascular reasons were 20.2% and 15.4%, respectively, in the main study, and 18.7% and 16.3%, respectively, in the 382 patients in the substudy. The three cardiac biomarkers were independently associated with the outcomes of hospitalization for any reason or for cardiovascular reasons, after adjustment for patient characteristics (Cox multivariate models, Table 4). MR-proADM and CT-proET-1 predicted hospitalization for any reason.

Discussion

Predicting recurrence of AF in patients in sinus rhythm with a history of AF is a difficult task [23]. Few prospective studies to address this issue have identified a limited set of clinical variables [24]. Amongst the biomarkers tested in small groups of patients, C-reactive protein, brain natriuretic peptide and NT-proBNP were found to predict recurrence of AF in patients with comorbidities [3–7]. We showed in 382 patients, with 53% 1-year incidence of a first recurrence of AF, that: (i) several cardiovascular markers are elevated during episodes of AF, when compared to sinus rhythm; (ii) markers of cardiac injury and/or strain (hsTnT, MR-proANP and NT-proBNP) and endothelin (CT-proET-1) have a modest but statistically significant predictive power of recurrence of AF; (iii) the higher the concentration of NT-proBNP, the earlier the onset of AF; and (iv) changes over time in the concentration of one of these biomarkers (MR-proANP) were also associated with subsequent recurrence of AF.

To our knowledge, data on the prognostic value of circulating cardiac troponin assayed using a highly sensitive method and of a stable fragment of a precursor of atrial natriuretic peptide have not been previously reported in patients with a history of AF.

The finding that modestly elevated concentrations (in almost 50% of cases within the accepted normal range) of three specific cardiac markers and endothelin predict recurrence of AF suggests that even a subclinical chronic condition of cardiac strain/injury may influence AF recurrence. The use of angiotensin-converting enzyme inhibitors (53.9%) and β-blockers (29.8%) in these patients with hypertension (84.8%) and heart failure (11%) may have contributed to lowering the concentrations of natriuretic peptides [25, 26].

The finding that the more recent the last cardioversion, the higher the concentration of cardiac markers at randomization supports the association of the latter with cardiac stress induced by or facilitating AF. A pathophysiological difference between predictors of recurrent AF and markers of ongoing AF is suggested by more elevated concentrations of several markers during episodes of AF as shown by scheduled ECG recordings together with blood sampling at the 6- and 12-month visits. Endothelin-1 was found to be significantly more elevated in patients with chronic heart failure who had a new episode of AF in the subsequent 23 months in the Val-HeFT trial [11] and was found to play a role in AF pathogenesis [14, 15].

In general, either previous or concomitant AF at any time over 1 year of follow-up (Table 2) was associated with higher concentrations of cardiovascular biomarkers. This suggests that indeed neurohumoral activation induced/sustained by AF was still present after recent conversion to sinus rhythm. By contrast, the remarkable stability of the concentrations of markers

Table 4 *Univariate and multivariate Cox models for the first recurrence of AF, hospitalization for any reason or hospitalization for a cardiovascular event*

	First recurrence of AF				Hospitalization for any reason (69 events)				Hospitalization for a cardiovascular reason (57 events)			
	Univariate model		Multivariate model		Univariate model		Multivariate model		Univariate model		Multivariate model	
Marker	HR [95%CI]	*P*	HR [95%CI]	*P*	HR [95%CI]	*P*	HR [95%CI]	*P*	HR [95%CI]	*P*	HR [95%CI]	*P*
hsTnT	1.13 [1.02–1.25]	0.02	1.15 [1.04–1.28]	0.007	1.21 [1.05–1.38]	0.007	1.21 [1.05–1.39]	0.007	1.22 [1.05–1.41]	0.007	1.19 [1.01–1.39]	0.03
NT-proBNP	1.21 [1.09–1.34]	0.0005	1.24 [1.11–1.39]	0.0001	1.45 [1.28–1.65]	<0.0001	1.49 [1.30–1.71]	<0.0001	1.49 [1.31–1.69]	<0.0001	1.46 [1.26–1.70]	<0.0001
MR-proANP	1.12 [0.99–1.28]	0.07	1.15 [1.01–1.30]	0.04	1.59 [1.33–1.92]	<0.0001	1.50 [1.25–1.81]	<0.0001	1.63 [1.34–1.99]	<0.0001	1.47 [1.19–1.81]	0.0004
MR-proADM	1.05 [0.92–1.20]	0.49	1.09 [0.95–1.26]	0.22	1.30 [1.08–1.58]	0.007	1.27 [1.05–1.55]	0.02	1.24 [0.99–1.54]	0.06	1.15 [0.91–1.45]	0.25
CT-proET-1	1.12 [0.98–1.28]	0.10	1.16 [1.01–1.33]	0.03	1.25 [1.06–1.48]	0.007	1.23 [1.03–1.46]	0.02	1.25 [1.04–1.50]	0.02	1.14 [0.89–1.45]	0.30
Copeptin	0.97 [0.84–1.11]	0.63	0.99 [0.86–1.14]	0.92	1.13 [0.91–1.40]	0.26	1.08 [0.87–1.35]	0.47	1.18 [0.94–1.47]	0.16	1.07 [0.84–1.37]	0.56

NT-proBNP, N-terminal probrain natriuretic peptide; MR-proANP, mid-regional proatrial natriuretic peptide; MR-proADM, mid-regional proadrenomedullin; hsTnT, high-sensitivity cardiac troponin T; CT-proET-1, C-terminal proendothelin-1; AF, atrial fibrillation.

Data shown as hazard ratio (HR) for an increment of 1 SD of baseline concentration of biomarkers. Clinical covariates were selected by stepwise procedures ($P < 0.05$) for each outcome. Significant covariates were as follows: (i) qualifying history of AF, lone AF with left atrial dilatation, documented coronary artery disease and smoking for first recurrence of AF; (ii) history of hypertension and systolic blood pressure for hospitalization for any reason; and (iii) history of hypertension, diabetes or peripheral artery disease, systolic blood pressure, smoking and QRS interval >120 ms for hospitalization for cardiovascular reasons.

in patients in sinus rhythm supports the lack of biohumoral activation in these patients.

Several studies have shown elevations of cardiac biomarkers in patients with AF and subsequent decreases once sinus rhythm has been restored [2, 4, 27, 28], but the underlying pathophysiology may be different in the case of recurrence of AF in patients in sinus rhythm with a history of AF, as in the present study. Although only markers of cardiac strain/injury were found to have predictive value, MR-proANP that is considered to be a more specific marker of atrial remodelling [29] showed a predictive power as modest as that of a 'ventricular' marker such as NT-proBNP.

Study limitations

This study enrolled mostly (84.8%) hypertensive patients with other diseases being under-represented; thus, the possible effects of comorbidities on the predictive power of these biomarkers cannot be assessed. Although the number of patients was larger than in any other published study in a similar setting, we may still have missed a relationship between biomarkers and outcomes. We estimated that we had at least 80% power (at $\alpha = 0.05$) to detect an effect size of 1.34, 1.39 and 1.38 (expressed as HR) for >median versus ≤median NT-proBNP, hsTnT and MR-proANP, respectively, and first recurrence of AF. Indeed, the relatively weak association of these biomarkers to risk of first recurrence of AF, as evidenced by Cox multivariate analysis and by low AUROC values, is not surprising because of the scarcity of predictive markers (either clinical or instrumental) in this field, and the concentrations of these biomarkers in our patients being in the normal range or only slightly elevated. The fact that risk of hospitalization for any cause or for cardiovascular reasons was predicted by the three cardiac markers supports the internal consistency of the sample studied. In other words, the sample size appears to be adequate to detect a predictive role of the cardiac biomarkers for clinical events, even at almost normal baseline levels.

Conclusions

Cardiovascular biomarkers are weakly associated with recurrent AF in patients in sinus rhythm who had either two or more documented episodes of AF in the previous 6 months or successful cardioversion for AF in the previous 2 weeks. Considering that the number of patients studied was higher than in most previously published studies to date, the weak predictive power observed is probably a better estimate than the previously reported more striking associations in smaller populations. The temporal relation between AF occurrence or recurrence and levels of biomarkers supports the pathophysiological consistency of the observed associations. Consistent with this is the paucity of predictors of AF recurrence, including ECG variables, in a post hoc analysis of all 1442 patients included in the GISSI-AF trial [30]. Even considering the number of patients and the duration of follow-up, the high incidence of outcome events (53.1%) in this study enabled identification of three cardiac markers for testing in future ad hoc studies in this relatively unexplored field.

Conflict of interest statement

Drs Latini and Masson have received honoraria and grant support from B.R.A.H.M.S. AG and Roche Diagnostics. Dr Zdunek is an employee of Roche Diagnostics. Dr Struck is an employee of B.R.A.H.M.S. AG, which holds patent rights to the markers. Drs. Latini, Masson, Barlera, Maggioni, Staszewsky, Franzosi and Disertori received institutional research support from Novartis Pharma. Reagents for measuring circulating biomarkers were kindly provided by B.R.A.H.M.S. AG and Roche Diagnostics. No other potential conflicts of interest relevant to this article were reported.

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Appendix

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