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Combination of Circulating Type I Collagen-Related Biomarkers Is Associated With Atrial Fibrillation

Susana Ravassa, PhD,[a,b,*] Gabriel Ballesteros, MD,[c,*] Begoña López, PhD,[a,b] Pablo Ramos, MD,[c] Jean Bragard, PhD,[d] Arantxa González, PhD,[a,b] María U. Moreno, PhD,[a,b] Ramón Querejeta, MD, PhD,[e] Enrique Vives, MD,[c] Ignacio García-Bolao, MD, PhD,[c] Javier Díez, MD, PhD[a,b,c,f]

ABSTRACT

BACKGROUND A combination of circulating biomarkers associated with excessive myocardial collagen type-I cross-linking or CCL+ (i.e., decreased carboxy-terminal telopeptide of collagen type-I to matrix metalloproteinase-1 ratio) and with excessive myocardial collagen type-I deposition or CD+ (i.e., increased carboxy-terminal propeptide of procollagen type-I) has been described in heart failure (HF) patients and associates with poor outcomes.

OBJECTIVES The purpose of this study was to investigate whether the CCL+CD+ combination of biomarkers associates with atrial fibrillation (AF).

METHODS Biomarkers were analyzed in serum samples from 242 HF patients (study 1) and 150 patients referred for AF ablation (study 2). Patients were classified into 3 groups (CCL−CD−, CCL+CD− or CCL−CD+, and CCL+CD+) in accordance to biomarker threshold values. Left atrial electroanatomic high-density mapping was performed in 71 patients from study 2.

RESULTS In study 1, 53.7% patients had AF at baseline and 19.6% developed AF (median follow-up 5.5 years). Adjusted odds and hazard ratios associated with baseline and new-onset AF, respectively, were both ≥3.3 ($p \leq 0.050$) in CCL+CD+ patients compared with CCL−CD− patients, with nonsignificant changes in the other group. In study 2, 29.3% patients had AF recurrence during 1-year post-ablation. The adjusted hazard ratio for AF recurrence was 3.4 ($p = 0.008$) in CCL+CD+ patients compared with CCL−CD− patients, with nonsignificant changes in the other group. The CCL+CD+ combination added incremental predictive value over relevant covariables. CCL+CD+ patients exhibited lower left atrial voltage than the remaining patients ($p = 0.005$).

CONCLUSIONS A combination of circulating biomarkers reflecting excessive myocardial collagen type-I cross-linking and deposition is associated with higher AF prevalence, incidence, and recurrence after ablation.
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From the [a]Program of Cardiovascular Diseases, CIMA, University of Navarra, and IdiSNA, Navarra Institute for Health Research, Pamplona, Spain; [b]CIBERCV, Carlos III Institute of Health, Madrid, Spain; [c]Department of Cardiology and Cardiac Surgery, University of Navarra Clinic, Pamplona, Spain; [d]Department of Physics & Applied Math, University of Navarra, Pamplona, Spain; [e]Donostia University Hospital, University of the Basque Country, and Biodonostia Research Institute, San Sebastián, Spain; and the [f]Department of Nephrology, University of Navarra Clinic, Pamplona, Spain. *Drs. Ravassa and Ballesteros contributed equally to this work. This study was supported by the Ministry of Economy and Competitiveness (Instituto de Salud Carlos III grants CB16/11/00483 and PI15/01909 cofinanced by FEDER funds, and SAF2014-58286-C2-2-R), the European Commission FP7 Programme (HOMAGE project 2012-305507), and the ERA-CVD Joint Transnational Call 2016 LYMIT-DIS (AC16/00020) and LIPCAR-HF (AC16/00016). Dr. García-Bolao has received consultant, proctoring, and speaker fees from Boston Scientific and St. Jude Medical. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Atrial fibrillation (AF) is an evolving epidemic responsible for substantial morbidity, mortality, and health care expenditure (1–3). In particular, when AF and heart failure (HF) occur in combination, clinical evolution is particularly poor (4). Left atrial (LA) myocardial interstitial fibrosis (MIF) is the hallmark lesion of the structural remodeling in AF and considered as the main substrate for AF perpetuation (5–7). In addition, MIF has been linked to lower effectiveness of AF catheter ablation (5). The identification of circulating biomarkers related to MIF, as an affordable and minimally invasive approach, is considered a potentially useful tool to detect patients at risk of AF, as well as to monitor the response to therapy in patients with AF (8).

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Cumulating evidence suggests that the detrimental pathophysiological impact of left ventricular (LV) MIF is related to alterations in both the quality (i.e., degree of cross-linking among collagen fibrils and type of collagen fibers) and the quantity (i.e., extent of collagen fiber deposition) of fibrotic tissue (9). For instance, it has been reported that patients with hypertensive heart disease and HF with both increased LV myocardial collagen type I cross-linking (CCL+) and increased myocardial collagen type I deposition (CD+) present with more severe cardiac dysfunction than HF patients with either or none of these alterations, independently of potential confounding factors (10). Some recently identified circulating biomarkers related to collagen type I metabolism have been found to associate with collagen type I cross-linking and deposition. On the one hand, the ratio of serum carboxy-terminal telopeptide of collagen type I to serum matrix metalloproteinase-1 (serum CITP:MMP-1 ratio) has been shown to be inversely correlated with LV myocardial collagen type I cross-linking, as the higher is the cross-linking among collagen type I fibrils, the lower will be the cleavage of CITP by MMP-1 during the process of degradation of the fiber (11). On the other hand, the serum carboxy-terminal propeptide of procollagen type I (PICP), released during the conversion of procollagen type I into fibril-forming mature collagen type I, has been reported to be directly correlated with LV myocardial collagen type I deposition (12). Interestingly, it has been shown that the combination of decreased serum CITP:MMP-1 ratio and increased serum PICP identifies HF patients that at the LV myocardial level present a pattern of complex MIF characterized by both increased collagen type I cross-linking and deposition (i.e., patients with the CCL+CD+ combination of biomarkers) (10). Importantly, HF patients with the biomarker combination are at higher risk of adverse outcomes than HF patients without the combination (10), this association being independent of potential confounding factors.

As both increased collagen cross-linking and deposition have been found in LA myocardium in AF patients (13) and AF is associated with fibrotic processes both in atria and ventricles (5), this study was designed to investigate whether the CCL+CD+ combination of biomarkers is associated with AF using 2 complementary studies by analyzing the association of the combination with the prevalence of AF and the incidence of new-onset AF in HF patients (study 1), and by evaluating the relationship of the combination with AF recurrence in patients referred for an AF ablation procedure (study 2). In addition, the association of the CCL+CD+ combination of biomarkers with LA electrical remodeling, as assessed by electroanatomic high-density mapping, was evaluated in a subgroup of patients submitted to AF ablation.

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

CCL = myocardial collagen type I cross-linking

CCL+ = excessive myocardial collagen type I cross-linking (i.e., decreased CITP:MMP-1 ratio)

CD = myocardial collagen type I deposition

CD+ = excessive myocardial collagen type I deposition (i.e., increased PICP)

CITP:MMP-1 = carboxy-terminal telopeptide of collagen type I to matrix metalloproteinase-1 ratio

HF = heart failure

MIF = myocardial interstitial fibrosis

PICP = carboxy-terminal propeptide of procollagen type I

V_m = left atrial mean voltage (units mV)

METHODS

All subjects gave written informed consent to participate in the study, and the institutional review committees (CEIC Donostia University Hospital and CEIC University of Navarra Clinic) approved the study protocols. The study conformed to the principles of the Helsinki Declaration.

STUDY 1. Study population. Patients were consecutively enrolled from 2002 to 2010 in the Division of Cardiology at the Donostia University Hospital (San Sebastián, Spain). The patient population consisted of 242 patients, (mean age 72.8 years; range 31 to 90 years; 47.1% women) with a long-term history of arterial hypertension and hypertensive heart disease. All patients had a previous clinical diagnosis of chronic HF based on the presence of at least 1 major and 2 minor Framingham criteria, and the median time of HF diagnosis before inclusion in the study was 2.30 years (range 0.04 to 21.6 years). All patients had presented previously with at least 1 hospitalization for HF. Patients with cardiac valve disease, hypertrophic cardiomyopathy, and other types of structural cardiomyopathy as well as patients with any clinical evidence of ischemic heart disease (i.e., angina symptoms, previous acute coronary syndrome, any previous revascularization

TABLE 1 Clinical and Echocardiographic Characteristics of Heart Failure Patients Categorized According to the Absence or Presence of Atrial Fibrillation at Baseline

	All Patients (N = 242)	Atrial Fibrillation		p Value
		No (n = 112)	Yes (n = 130)	
Anthropometrics				
Age, yrs	72.8 ± 10.5	70.6 ± 11.0	74.7 ± 9.8	0.003
Male	128 (52.9)	57 (50.9)	71 (54.6)	0.56
BMI, kg/m^2	30.9 ± 5.2	31.2 ± 4.6	30.7 ± 5.8	0.48
SBP, mm Hg	149.0 ± 23.7	152.0 ± 25.6	146.0 ± 21.7	0.052
DBP, mm Hg	85.9 ± 12.7	86.1 ± 12.6	85.6 ± 12.8	0.81
HR, beats/min	78.2 ± 18.9	74.5 ± 15.4	81.4 ± 21.0	0.004
eGFR, ml/min/1.73 m^2	74.5 ± 29.0	77.9 ± 26.5	71.5 ± 30.8	0.09
Comorbidities				
Obesity	125 (51.7)	68 (60.7)	57 (43.8)	0.009
Hyperglycemia	33 (13.6)	13 (11.6)	20 (15.4)	0.39
Dyslipidemia	127 (52.5)	61 (54.5)	66 (50.8)	0.66
CKD	79 (32.6)	29 (25.9)	50 (38.5)	0.034
NYHA functional class				
II	100 (41.3)	47 (42.0)	53 (40.8)	
III	132 (54.6)	61 (54.4)	71 (54.6)	0.89
IV	10 (4.1)	4 (3.6)	6 (4.6)	
NT-proBNP, pg/ml	1,330 (877–2,055)	1,095 (750–1,952)	1,480 (1,028–2,164)	0.005
Duration of HF, yrs	2.1 (0.6–4.0)	2.1 (0.5–4.0)	2.1 (0.6–4.0)	0.97
Medical therapy				
ACE inhibitor/ARB	205 (84.7)	92 (82.1)	113 (86.9)	0.30
Diuretics	199 (82.2)	89 (79.5)	110 (84.6)	0.30
Beta-blockers	123 (50.8)	70 (62.5)	53 (40.8)	0.001
Calcium-channel blocker	45 (18.6)	14 (12.5)	31 (23.8)	0.024
MR blockers	35 (14.5)	13 (11.6)	22 (16.9)	0.24
Digoxin	106 (43.8)	40 (35.7)	66 (50.8)	0.019
Echocardiography				
LVMI, g/m^2	151 ± 59.4	146 ± 52.8	156 ± 64.7	0.23
RWT	0.45 ± 0.13	0.44 ± 0.11	0.45 ± 0.14	0.56
LVEDD, mm	51.0 ± 9.6	50.5 ± 9.2	51.4 ± 9.9	0.49
LVVI, ml/m^2	70.5 ± 31.6	69.1 ± 27.7	71.8 ± 34.7	0.94
LVEF, %	56.1 ± 16.0	56.5 ± 16.5	55.8 ± 15.6	0.74
LVEF <50%	78 (32.2)	37 (33.0)	41 (31.5)	0.80
E, cm/s	89.2 ± 27.2	80.7 ± 27.0	96.2 ± 25.4	<0.0001
E:A ratio	0.93 ± 0.41	0.89 ± 0.34	1.06 ± 0.58	0.32
IVRT, ms	108 ± 26.0	115 ± 25.4	102 ± 25.2	<0.0001
DT, ms	210 ± 56.0	231 ± 59.0	192 ± 46.9	<0.0001
LA volume index, ml/m^2	41.6 ± 14.2	37.6 ± 14.1	44.9 ± 13.5	<0.0001
LA volume index >34 ml/m^2	174 (71.9)	64 (57.1)	110 (84.6)	<0.0001

Values are mean ± SD, n (%), or median (interquartile range). Obesity was defined as BMI >30 kg/m^2. Hyperglycemia was defined as plasma glucose >126 mg/dl. Dyslipidemia was defined by the presence of hypercholesterolemia (serum cholesterol >200 mg/dl) and/or hypertriglyceridemia (serum triglycerides >150 mg/dl). CKD was defined as eGFR <60 ml/min/1.73 m^2.

A = maximum late transmitral flow velocity in diastole; ACE = angiotensin-converting enzyme; ARB = angiotensin II type 1 receptor blocker; BMI = body mass index; CKD = chronic kidney disease; DBP = diastolic blood pressure; DT = deceleration time; E = maximum early transmitral flow velocity in diastole; eGFR = estimated glomerular filtration rate; HF = heart failure; HR = heart rate; IVRT = isovolumetric relaxation time; LA = left atrial; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVMI = left ventricular mass index; LVVI = left ventricular volume index; MR = mineralocorticoid receptor; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; RWT = relative wall thickness; SBP = systolic blood pressure.

procedure, ischemic signs in electrocardiogram, or pathological Q waves), diabetes mellitus, stages 3 to 5 chronic kidney disease (CKD), and regional wall motion abnormalities were excluded.

AF prevalence, whether paroxysmal or persistent, was diagnosed by electrocardiograms or Holter at (or upon) hospital admission and/or during the recruitment process. Patients were considered to have had AF if at least 1 episode of this cardiac arrhythmia lasting longer than 30 s was documented, as is usual practice.

Cardiac evaluation. Two-dimensional echocardiographic pulsed Doppler imaging was performed in all patients. LV mass and dimensions and parameters assessing systolic and diastolic function were measured. For further details, please see the Online Appendix.

Study outcome. The study outcome was the first electrocardiogram-documented AF episode, identified by medical record review. Vital status was ascertained by Social Security Medical Registries, and in the cases where any patient failed to appear at the scheduled review, his/her relatives were contacted by phone. Two board-certified cardiologist investigators, blinded to the patients' clinical echocardiographic and biochemical data, adjudicated the outcome according to the pre-specified criteria.

STUDY 2. Study population. The 150 consecutive patients who underwent ablation of paroxysmal or persistent symptomatic AF were prospectively enrolled from 2015 to 2017 in the Department of Cardiology and Cardiovascular Surgery at the University of Navarra Clinic (Pamplona, Spain). In a subgroup of 71 patients, a detailed high-density voltage map of the entire left atrium (HD_VM) was performed by the same principal operator, as previously described (14,15), for correlation of mean voltage and low voltage area with the CCL+CD+ combination pattern. If the patient was in AF before the procedure, electrical cardioversion was performed before starting the intervention; thus, all maps were performed in sinus rhythm. For further details on map building, ablation strategy and quantitative map analyses see the Online Appendix.

Study outcome. Patients were followed-up in the outpatient clinic of the University of Navarra Clinic according to a standard clinical practice. Follow-up visits including 12-lead ECG and 24-h Holter ECG were scheduled 3, 6, and 12 months after discharge and additional nonscheduled visits were performed if patients presented with any symptoms suggestive of AF. Episodes of AF or other atrial arrhythmias lasting longer than 30 s were considered for analysis. Episodes that occurred after ablation, with a blanking period of the first 3 months, were

considered to indicate AF recurrence. Antiarrhythmic drugs were usually continued for the first 3 months after ablation and then systematically discontinued.

BIOCHEMICAL STUDIES. In patients from both studies, serum PICP, CITP, MMP-1, and the amino-terminal pro-B-type natriuretic peptide (NT-proBNP) were measured by ELISA as previously described. For further details please see the Online Appendix.

BIOMARKER-BASED CLASSIFICATION OF PATIENTS. As previously established (11), patients were classified as CCL+ (with increased myocardial collagen type I cross-linking) or CCL− (without increased myocardial collagen type I cross-linking) according to the values of serum CITP:MMP-1 ratio below or above 1.968, respectively. Further, as previously established (10), patients were also classified as CD+ (with severe myocardial collagen type I deposition) or CD− (without severe myocardial collagen type I deposition) according to the values of serum PICP above or below 110.8 ng/ml, respectively. Following these criteria, patients were classified in 3 groups reflecting the diverse combinations of biomarkers: CCL−CD−, CCL−CD+ or CCL+CD− and CCL+CD+.

STATISTICAL ANALYSIS. Normality was demonstrated by the Shapiro-Wilks or Kolmogorov-Smirnov tests. Nonparametric distributed variables were examined after logarithmic transformation. Differences between 2 groups of subjects were tested by Student's *t*-test for unpaired data once normality was demonstrated; otherwise, the nonparametric test (Mann-Whitney *U* test) was used. Baseline characteristics were compared between patients by using the chi-square test or Fisher exact test, when necessary, for categorical variables. Linear tests for trend were used to assess any tendencies across the different groups. Multivariable logistic regression models were used to assess the independent relationships after adjustment for relevant covariates selected by a step-down procedure removing the least significant covariable at each step until all p values of covariables were <0.15. Multicollinearity was evaluated by examining the variance inflation factor. Calibration of the logistic models was assessed using the Homer-Lemeshow goodness-of-fit test.

Time to new-onset AF was plotted using Kaplan-Meier curves for patients stratified in the diverse groups, with statistical significance assessed using the long-rank test. Patients without outcome were censored at the date of their last follow-up. Fine-Gray competing risk models were used to obtain the sub-hazard ratios for de novo incidence of AF, considering death as a competing event. Time to recurrence of AF was plotted using Kaplan-Meier curves (with a blanking period of 3 months following ablation applied) with statistical significance assessed using

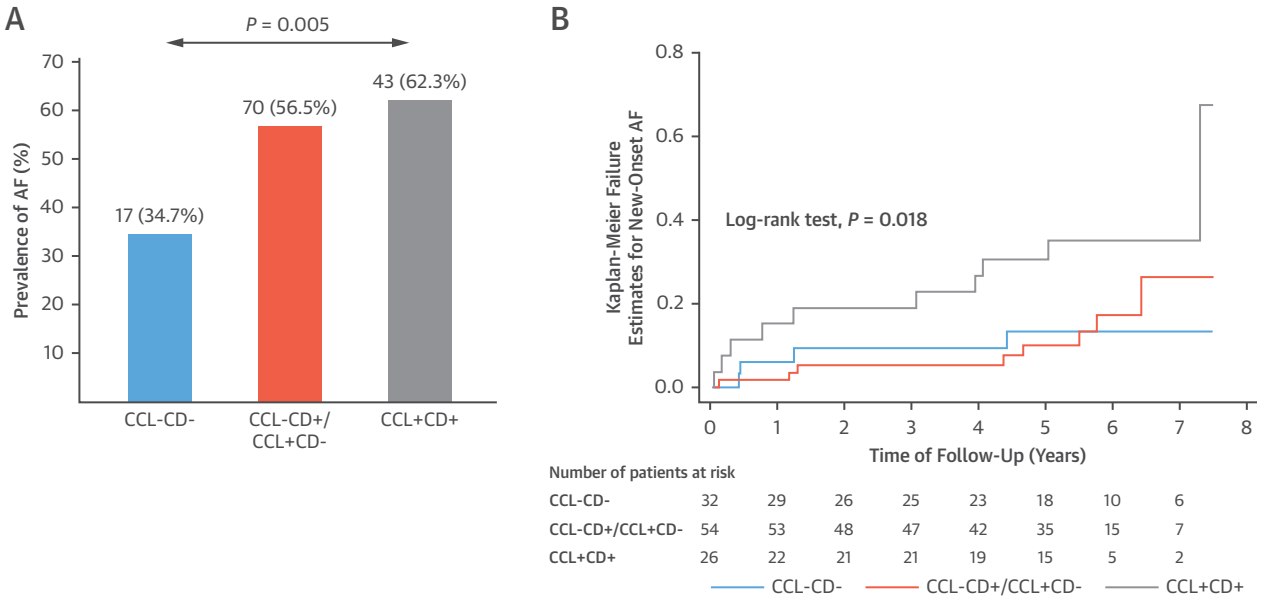
TABLE 2 Clinical and Echocardiographic Characteristics of Heart Failure Patients Categorized According to the Presence or Absence of High CCL and Severe CD

	CCL−CD− (n = 49)	CCL−CD+ or CCL+CD− (n = 124)	CCL+CD+ (n = 69)	p Value for Trend
Anthropometrics				
Age, yrs	69.9 ± 11.7	73.5 ± 10.7	73.7 ± 9.0	0.07
Male	25 (51.0)	68 (54.8)	35 (50.7)	0.82
BMI, kg/m^2	31.1 ± 4.7	31.1 ± 5.4	30.5 ± 5.4	0.51
SBP, mm Hg	153.0 ± 28.4	149.0 ± 22.6	147.0 ± 21.8	0.14
DBP, mm Hg	87.3 ± 12.9	85.0 ± 12.8	86.3 ± 12.4	0.77
HR, beats/min	77.9 ± 21.3	78.2 ± 17.6	78.4 ± 19.7	0.91
eGFR, ml/min/1.73 m^2	77.8 ± 29.2	73.7 ± 29.9	73.6 ± 27.4	0.50
Comorbidities				
Obesity	28 (57.1)	64 (51.6)	33 (47.8)	0.32
Hyperglycemia	6 (12.2)	20 (16.1)	7 (10.1)	0.64
Dyslipidemia	29 (59.2)	68 (54.8)	30 (43.5)	0.11
CKD	15 (30.6)	42 (33.9)	22 (31.9)	0.92
NYHA functional class				
II	21 (42.9)	56 (45.2)	23 (33.3)	
III	28 (57.1)	63 (50.8)	41 (59.4)	0.08
IV	0 (0.0)	5 (4.0)	5 (7.2)	
NT-proBNP, pg/ml	1,090 (709–1,814)	1,287 (869–2,119)	1,601 (1,043–2,221)	0.002
Duration of HF, yrs	2.5 (0.8–4.1)	2.0 (0.5–4.0)	2.0 (0.6–4.0)	0.64
Medical therapy				
ACE inhibitor/ARB	45 (91.8)	100 (80.6)	60 (87.0)	0.62
Diuretics	39 (79.6)	103 (83.1)	57 (82.6)	0.71
Beta-blockers	29 (59.2)	64 (51.6)	30 (43.5)	0.09
Calcium-channel blocker	9 (18.4)	26 (21.0)	10 (14.5)	0.52
MR blockers	5 (10.2)	22 (17.7)	8 (11.6)	0.98
Digoxin	14 (28.6)	61 (49.2)	31 (44.9)	0.12
Echocardiography				
LVMI, g/m^2	138 ± 58.0	152 ± 56.9	160 ± 63.7	0.034
RWT	0.43 ± 0.12	0.46 ± 0.12	0.44 ± 0.15	0.96
LVEDD, mm	50.1 ± 8.1	50.4 ± 9.1	52.7 ± 11.2	0.13
LVVI, ml/m^2	65.3 ± 24.0	68.9 ± 28.4	77.0 ± 39.9	0.042
LVEF, %	55.3 ± 14.5	58.3 ± 15.6	52.9 ± 17.4	0.74
LVEF <50%	16 (32.7)	34 (27.4)	28 (40.6)	0.25
E, cm/s	81.6 ± 26.0	91.8 ± 27.3	89.1 ± 27.2	0.42
E:A ratio	0.93 ± 0.40	0.95 ± 0.38	0.83 ± 0.30	0.33
IVRT, ms	109 ± 22.3	106 ± 29.0	109 ± 23.0	0.96
DT, ms	228 ± 57.2	208 ± 55.2	199 ± 54.2	0.009
LA volume index, ml/m^2	40.8 ± 15.0	42.5 ± 15.2	40.9 ± 12.0	0.97
LA volume index >34 ml/m^2	32 (65.3)	90 (72.6)	52 (75.4)	0.25

Values are mean ± SD, n (%), or median (interquartile range). Obesity was defined as BMI >30 kg/m^2. Hyperglycemia was defined as plasma glucose >126 mg/dl. Dyslipidemia was defined by the presence of hypercholesterolemia (serum cholesterol >200 mg/dl) and/or hypertriglyceridemia (serum triglycerides >150 mg/dl). CKD was defined as eGFR <60 ml/min/1.73 m^2.

CCL = collagen cross-linking; CD = collagen deposition; other abbreviations as in Table 1.

FIGURE 1 Prevalence and Incidence of AF in Patients With Heart Failure Classified According to Study Subgroups



Percentage of AF at baseline **(A)** and Kaplan-Meier curves for the incidence of new-onset AF **(B)** in patients with heart failure classified in 3 groups according to the presence (+) or absence (−) of increased CCL and increased CD, based on the serum carboxy-terminal telopeptide of collagen type I to matrix metalloproteinase-1 ratio and the serum carboxy-terminal propeptide of procollagen type I threshold values, respectively, as defined in the main text. AF = atrial fibrillation; CCL = collagen type I cross-linking; CD = collagen type I deposition.

the long-rank test. A multiple Cox regression analysis was used to calculate hazard ratios for the risk of future AF recurrence, adjusting for covariables. For all longitudinal analyses, adjustment for relevant covariables with p values <0.1 in univariate analyses were performed. The proportional subhazard and hazard assumptions were verified using Schoenfeld's residuals for each model.

The additional value of the combination of biomarkers for risk prediction of AF recurrence was assessed with Harrell's C statistics and the continuous net reclassification index (NRI) index. The variance for the Harrell's C estimates were calculated using the jackknife approach with the Stata package "somersd." The variances for the NRI estimates were calculated using bootstrapping (1,000 resamples) with the Stata command "incrisk."

Values are expressed as mean ± SD or median (interquartile range), and categorical variables as numbers and percentages. Statistical significance was set as a 2-sided p value of 0.05. The statistical analyses were performed by using SPSS version 15.0 (IBM, Armonk, New York) and STATA version 12.1 (StataCorp, College Station, Texas) software.

RESULTS

STUDY 1. Prevalence of AF. A total of 130 (53.7%) HF patients had AF at baseline. The clinical and echocardiographic characteristics of HF patients classified according to the absence or presence of AF are shown in Table 1.

Taking into account the criteria previously mentioned in the Methods section on biomarker-based classification, 49 (20.2%) patients were categorized as CCL−CD−, 124 (51.2%) as either CCL−CD+ or CCL+CD−, and 69 (28.5%) as CCL+CD+. The clinical and echocardiographic characteristics of HF patients classified according to this categorization are shown in Table 2. NT-proBNP, LV mass index, and LA volume index significantly increased, and deceleration time significantly decreased, along the CCLCD groups (p value for trend ≤0.042). The prevalence of AF progressively increased along the CCLCD groups (chi-square = 8.01; p = 0.005) (Figure 1A). In multivariable-adjusted analyses accounting for age, heart rate, New York Heart Association functional class, estimated glomerular filtration rate <60 ml/min/1.73 m^2, LA volume >34 ml/m^2, deceleration time, NT-proBNP

(log) and treatment with beta-blockers, calcium antagonists, and digoxin, CCL+CD+ patients exhibited a 3-fold higher odds of AF compared with patients with CCL−CD− patients as the reference group (CCL+CD+: odds ratio: 3.32 [95% confidence interval (CI): 1.25 to 8.83]; p = 0.016; CCL−CD+ or CCL+CD−: odds ratio: 2.38 [95% CI: 1.00 to 5.65]; p = 0.049).

Incidence of new-onset AF. A total of 112 patients with HF did not have a previous history of AF. Of these, 22 (19.6%) developed new-onset AF during a median follow-up of 5.5 years (range 0.2 to 7.5 years). The baseline clinical and echocardiographic characteristics of these patients classified according to the occurrence of new-onset AF are shown in Table 3.

According to the biomarker-based classification, 32 (28.6%) patients were categorized as CCL−CD−, 54 (48.2%) as either CCL−CD+ or CCL+CD−, and 26 (23.2%) as CCL+CD+. There was a progressive increment of new-onset AF along the CCLCD groups (log-rank test: chi-square = 8.1; p = 0.018) (Figure 1B). Unadjusted analyses, accounting for all-cause death as a competing risk, showed a significantly increased risk of new-onset incidence of AF in CCL+CD+ patients with a hazard ratio of 3.62 (95% CI: 1.11 to 11.7; p = 0.032) compared with CCL−CD− patients, which remained significant after adjustment for covariables with p values <0.1 in univariate analysis (age and LV ejection fraction) (Online Table 1) (adjustment for age: 3.27 [95% CI: 1.00 to 10.8]; p = 0.050; adjustment for LVEF: 3.55 [95% CI: 1.08 to 11.7]; p = 0.037) (Online Table 2). In addition, adjustment for LA volume index rendered a hazard ratio of 3.57 (95% CI: 1.10 to 11.6; p = 0.034) in CCL+CD+ patients compared with CCL−CD− patients (Online Table 2).

STUDY 2. Recurrence of AF. A total of 150 patients with paroxysmal or persistent AF underwent pulmonary vein isolation. Of these, in a subgroup of 71 patients (47.3%), a detailed high-density voltage map of the entire left atrium (HD_VM) was performed. At 12-month follow-up, 44 (29.3%) patients showed AF recurrence.

Table 4 shows the baseline clinical and LA characteristics in all patients and in patients classified according to AF recurrence during 1 year after ablation procedure. In accordance with the biomarker-based classification, 44 (29.3%) patients were categorized as CCL−CD−, 63 (42.0%) as either CCL−CD+ or CCL+CD−, and 43 (28.7%) as CCL+CD+. The clinical and LA characteristics of patients classified according to this categorization are shown in Table 5. LA volume index progressively increased along the CCLCD groups (p for trend = 0.016). In addition, AF recurrence progressively increased along the CCLCD groups, with 7 (15.9%), 15 (23.8%), and 22 (51.2%) AF recurrences in the CCL−CD−, CCL−CD+ or

TABLE 3 Clinical and Echocardiographic Characteristics of Heart Failure Patients Categorized According to the De Novo Incidence of Atrial Fibrillation

	Incidence of Atrial Fibrillation		
	No (n = 90)	Yes (n = 22)	p Value
Anthropometrics			
Age, yrs	70.0 ± 11.2	73.3 ± 9.9	0.21
Male	47 (52.2)	10 (45.5)	0.57
BMI, kg/m^2	31.4 ± 4.7	30.4 ± 4.0	0.39
SBP, mm Hg	152.0 ± 25.4	152.0 ± 27.0	0.99
DBP, mm Hg	85.5 ± 11.5	88.4 ± 16.5	0.44
HR, beats/min	74.5 ± 15.1	74.7 ± 16.8	0.96
eGFR, ml/min/1.73 m^2	79.6 ± 25.7	70.9 ± 28.9	0.17
Comorbidities			
Obesity	56 (62.2)	12 (54.5)	0.51
Hyperglycemia	9 (10.0)	4 (18.2)	0.28
Dyslipidemia	51 (56.7)	10 (45.5)	0.37
CKD	21 (23.3)	8 (36.4)	0.20
NYHA functional class			
II	39 (43.3)	8 (36.4)	
III	49 (54.4)	12 (54.5)	0.27
IV	2 (2.2)	2 (9.1)	
NT-proBNP, pg/ml	1,095 (777–1,875)	1,339 (663–2,259)	0.54
Duration of HF, yrs	2.1 (0.8–4.0)	2.2 (0.2–4.4)	0.70
Medical therapy			
ACE inhibitor/ARB	73 (81.1)	19 (86.4)	0.56
Diuretics	72 (80.0)	17 (77.3)	0.78
Beta-blockers	58 (64.4)	12 (54.5)	0.39
Calcium-channel blocker	13 (14.4)	1 (4.5)	0.21
MR blockers	11 (12.2)	2 (9.1)	0.68
Digoxin	33 (36.7)	7 (31.8)	0.67
Echocardiography			
LVMI, g/m^2	145 ± 51.5	154 ± 58.5	0.57
RWT	0.44 ± 0.11	0.45 ± 0.11	0.66
LVEDD, mm	50.7 ± 9.5	49.7 ± 8.1	0.64
LVVI, ml/m^2	69.5 ± 28.1	67.4 ± 26.6	0.76
LVEF, %	58.2 ± 16.5	49.8 ± 15.3	0.033
LVEF <50%	26 (28.9)	11 (50.0)	0.06
E, cm/s	81.9 ± 28.1	75.1 ± 21.1	0.36
E:A ratio	0.90 ± 0.33	1.03 ± 0.79	0.97
IVRT, ms	114 ± 25.5	117 ± 25.7	0.57
DT, ms	233 ± 56.6	221 ± 69.2	0.41
LA volume index, ml/m^2	36.6 ± 13.0	41.7 ± 17.5	0.13
LA volume index >34 ml/m^2	50 (55.6)	14 (63.6)	0.49

Values are mean ± SD, n (%), or median (interquartile range). Obesity was defined as BMI >30 kg/m^2. Hyperglycemia was defined as plasma glucose >126 mg/dl. Dyslipidemia was defined by the presence of hypercholesterolemia (serum cholesterol >200 mg/dl) and/or hypertriglyceridemia (serum triglycerides >150 mg/dl). CKD was defined as eGFR <60 ml/min/1.73 m^2.

Abbreviations as in Table 1.

TABLE 4 Baseline Clinical Characteristics In Patients Classified According to the Recurrence of Atrial Fibrillation After Ablation Procedure

		Response After Ablation Procedure		
	All Patients (N = 150)	No Recurrence (n = 106)	Recurrence (n = 44)	p Value
Age, yrs	64.2 ± 9.5	63.3 ± 9.3	66.4 ± 9.9	0.07
Male	101 (67.3)	75 (70.8)	26 (59.1)	0.17
BMI, kg/m^2	27.4 ± 3.7	27.2 ± 3.7	27.8 ± 3.6	0.35
SBP, mm Hg	123.0 ± 17.7	122.0 ± 16.7	125.0 ± 19.8	0.36
DBP, mm Hg	76.4 ± 12.9	75.9 ± 12.0	77.8 ± 14.8	0.39
eGFR, ml/min/1.73 m^2	76.5 ± 19.6	77.5 ± 19.3	74.2 ± 20.5	0.49
Treatment				
Antiarrhythmic	65 (43.3)	42 (39.6)	23 (52.3)	0.16
Calcium antagonists/BB	82 (54.7)	55 (51.9)	27 (61.4)	0.29
Statins	49 (32.7)	31 (29.2)	18 (40.9)	0.16
ACE inhibitor/ARB	69 (46.0)	51 (48.1)	18 (40.9)	0.37
Diuretics	28 (18.7)	16 (15.1)	12 (27.3)	0.09
Comorbidities				
Obesity	30 (20.0)	20 (18.9)	10 (22.7)	0.55
Hypertension	85 (56.7)	60 (56.6)	25 (56.8)	0.98
Dyslipidemia	67 (44.7)	43 (40.6)	24 (54.5)	0.12
Diabetes	15 (10.0)	11 (10.4)	4 (9.1)	0.81
Sleep apnea	15 (10.0)	9 (8.5)	6 (13.6)	0.34
CPAP use	12 (8.0)	7 (6.6)	5 (11.4)	0.33
CKD	18 (12.0)	11 (10.4)	7 (15.9)	0.47
Medical history				
Cerebrovascular accident	9 (6.0)	8 (7.5)	1 (2.3)	0.22
Heart failure	13 (8.7)	8 (7.5)	5 (11.4)	0.45
Peripheral vascular disease	4 (2.7)	2 (1.9)	2 (4.5)	0.36
Ischemic cardiomyopathy	8 (5.3)	4 (3.8)	4 (9.1)	0.19
Neoplasia	9 (6.0)	7 (6.6)	2 (4.5)	0.63
Type of AF				
Paroxysmal	86 (57.3)	69 (65.1)	17 (38.6)	0.003
Persistent	64 (42.7)	37 (34.9)	27 (61.4)	
Duration of AF, months	24.0 (5.5-72.0)	24.0 (5.0-72.0)	28.0 (6.0-60.0)	0.99
EHRA score				
1	28 (18.7)	22 (20.8)	6 (13.6)	
2	87 (58.0)	60 (56.6)	27 (61.4)	0.59
3 and 4	35 (23.3)	24 (22.6)	11 (25.0)	
Computed tomography				
LA diameter, mm	61.3 ± 7.8	60.4 ± 7.4	63.3 ± 8.5	0.047
Conventional echocardiography				
LVEF, %	60.7 ± 8.2	60.2 ± 7.9	61.9 ± 9.1	0.29
LA volume index, ml/m^2	31.3 ± 12.8	29.4 ± 11.7	34.8 ± 14.0	0.022

Values are mean ± SD, n (%), or median (interquartile range). Obesity was defined as BMI >30 kg/m^2. Hypertension was defined as systolic blood pressure >139 mm Hg, diastolic blood pressure >89 mm Hg, or antihypertensive treatment. Dyslipidemia was defined by the presence of hypercholesterolemia (serum cholesterol >200 mg/dl) and/or hypertriglyceridemia (serum triglycerides >150 mg/dl). CKD was defined as eGFR <60 ml/min/1.73 m^2.

AF = atrial fibrillation; BB = beta-blockers; CPAP = positive pressure therapy; EHRA = European Heart Rhythm Association; other abbreviations as in Table 1.

CCL+CD−, and CCL+CD+ groups, respectively (log-rank test: chi-square = 14.5, p = 0.001) (**Figure 2**). As shown in **Table 6**, and in the Online Figure 1, the association of the CCL+CD+ combination of biomarkers with AF recurrence remained significant after adjustment for covariables with p values <0.1 in univariate analysis (Online Table 3): age, persistent AF, and LA volume index. Of note, the association of the CCL+CD+ combination of biomarkers with AF recurrence was not influenced by the presence of HF, with a hazard ratio of 3.78 (95% CI: 1.60 to 8.92; p = 0.002) compared with the CCL−CD− group.

Harrell's C statistics show that prediction of AF recurrence was improved after adding the CCL+CD+ combination of biomarkers to the basal model including age, persistent AF, and LA volume index as relevant clinical covariables associated with AF recurrence, with p values <0.1 in univariate analysis (Online Table 3) (Basal model: area under the curve = 0.676 [95% CI: 0.570 to 0.782] vs. basal model plus biomarkers of CCL+ and CD+: area under the curve = 0.767 [95% CI: 0.679 to 0.855]; p = 0.049) (Online Figure 2). In addition, the continuous NRI indicated that the addition of this phenotype to the basal model improved risk prediction of AF recurrence (**Table 6**).

Analyses of the HD$_V$M maps. In the 71 patients who underwent pulmonary vein isolation guided by the HD$_V$M system, high-resolution LA electroanatomic maps were created, containing 12,501 ± 5,613 valid voltage points per map. After creation of the voltage maps, the mean voltage (V$_m$), representing the spatial average voltage of the left atrium, was calculated using a novel computer algorithm as explained in the Methods section. In addition, the percentage of scar area as defined by a voltage <0.5 mV (16,17) was estimated. According to the biomarker-based classification, 16 (22.5%) patients were classified as CCL+CD+, and 55 (77.5%) patients as either CCL−CD− or CCL−CD+ or CCL+CD−. As shown in **Figure 3A**, CCL+CD+ patients exhibited lower V$_m$ compared with the remaining patients (0.75 ± 0.39 mV vs. 1.15 ± 0.67 mV; p = 0.005). As shown in **Figure 3B**, CCL+CD+ patients exhibited a higher percentage of area <0.5 mV compared with the remaining patients (0.63 ± 0.10% vs. 0.54 ± 0.17%; p = 0.033).

DISCUSSION

The main findings of this study are as follows. First, a combination of serum biomarkers associated with increased myocardial collagen type I cross-linking and deposition (i.e., the CCL+CD+ combination) is independently associated with the prevalence of AF and with the incidence of new-onset AF in HF patients. Second, the CCL+CD+ combination of biomarkers is independently associated with AF recurrence after ablation and predicts AF recurrence over and beyond clinically relevant variables. And

third, AF patients with the CCL+CD+ combination of biomarkers have lower LA V_m and a higher percentage of area with voltage values associated with the presence of fibrotic scars than AF patients without that combination.

It has been recently demonstrated that collagen turnover biomarkers, along with prothrombotic and inflammatory markers, are elevated in AF (18). In addition, a number of studies have investigated the association of altered circulating levels of biomarkers of MIF, including procollagen- and collagen-derived peptides, and profibrotic and antifibrotic factors, either with presence of AF or new-onset AF or with AF recurrence after percutaneous ablation or cardioversion. However, most of these studies were performed in small and heterogeneous AF populations, and did not assess the added incremental value of the biomarkers beyond relevant clinical variables (reviewed by Begg et al. [8]). In addition, for many of the investigated biomarkers, the histologically-proven evidence of their association with either atrial or ventricular MIF was lacking (19). The current study is the first to report that a combination of biomarkers is independently associated with the prevalence of AF, the incidence of new-onset AF, and post-ablation AF recurrence, the latter with added incremental value over and beyond relevant clinical variables. Although the 2 biomarkers included in the combination have been histologically related with MIF at the ventricular level (10-12), their association with atrial MIF has been previously reported only for one of them (serum PICP [20,21]) but not yet for the other (serum CITP:MMP-1 ratio). Nevertheless, although it does not confirm a causal relationship, it is worth mentioning that AF patients with the CCL+CD+ combination of biomarkers exhibit larger low LA voltage areas, an alteration that is universally considered a sign of fibrosis or scar (22) and that has been shown to be associated with LA fibrosis identified by magnetic resonance imaging (23,24), suggesting that this combination of biomarkers may be reflecting myocardial MIF, at not only the ventricular but also the atrial level.

The combination of biomarkers here identified sheds light on the pathophysiology of the association of MIF with AF, as it reinforces the notion that the complexity of MIF resulting from alterations in both the properties and the amount of the deposited collagen fibers seems to be relevant for this association (9). The properties of collagen deposits may be influenced, among other mechanisms, by increased lysyl oxidase (LOX)-mediated intermolecular covalent linkage or cross-linking of fibrils to form fibers that are highly resistant to degradation by MMPs and then accumulate forming extensive deposits of fibrotic tissue (9). In this regard, it has been shown that LA samples from patients with AF showed higher collagen content, collagen cross-linking, and LOX expression than samples from patients in sinus rhythm (13). In addition, the left atrium of transgenic mice with cardiac overexpression of Rac1 that develop AF at old age exhibited upregulation of LOX

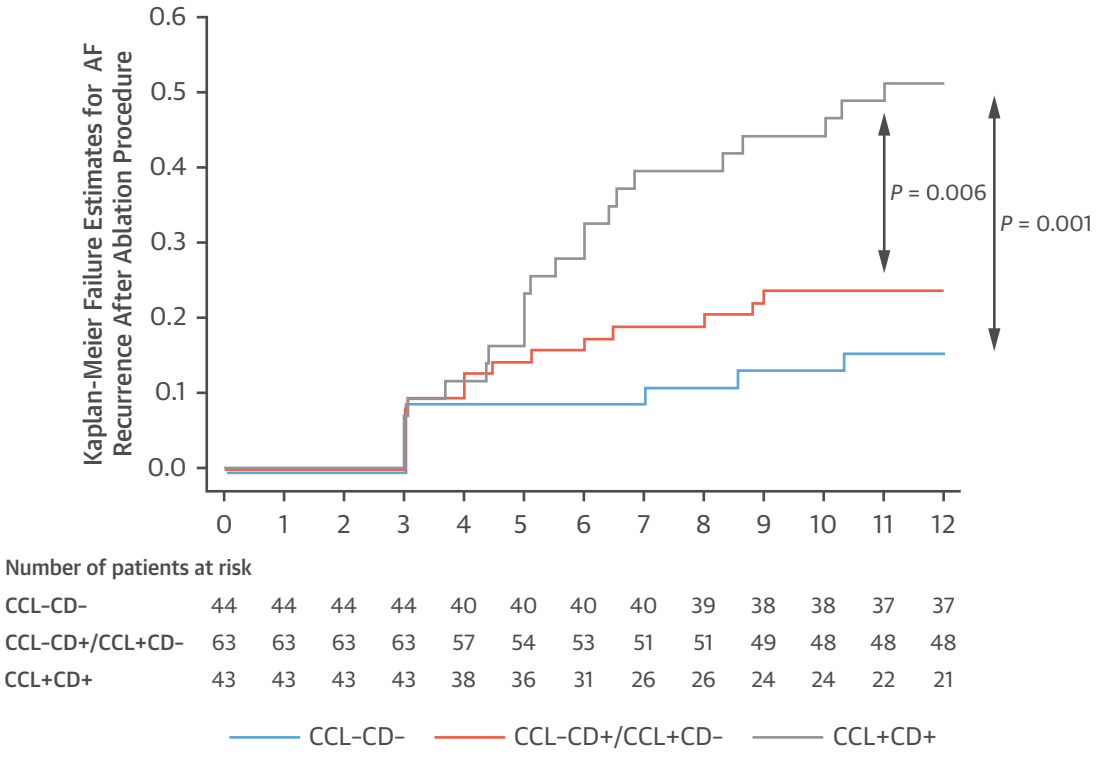
TABLE 5 Baseline Clinical Characteristics in Patients Referred for Atrial Fibrillation Ablation Categorized According to the Presence or Absence of High CCL and Severe CD

	CCL−CD− (n = 44)	CCL−CD+ or CCL+CD− (n = 63)	CCL+CD+ (n = 43)	p Value for Trend
Age, yrs	63.7 ± 8.9	65.8 ± 10.1	62.5 ± 9.3	0.58
Male	30 (68.2)	41 (65.1)	30 (69.8)	0.88
BMI, kg/m^2	28.2 ± 3.7	26.7 ± 3.4	27.5 ± 3.9	0.36
SBP, mm Hg	126.0 ± 21.3	122.0 ± 17.0	122.0 ± 14.6	0.33
DBP, mm Hg	76.9 ± 13.8	75.4 ± 13.4	77.5 ± 11.0	0.84
eGFR, ml/min/1.73 m^2	78.9 ± 21.1	74.6 ± 18.0	76.9 ± 20.1	0.72
Treatment				
Antiarrhythmic	18 (40.9)	27 (42.9)	20 (46.5)	0.60
Calcium antagonists/BB	22 (50.0)	33 (52.4)	27 (62.8)	0.23
Statins	13 (29.5)	23 (36.5)	13 (30.2)	0.99
ACE inhibitor/ARB	20 (45.5)	33 (52.4)	16 (37.2)	0.33
Diuretics	9 (20.5)	11 (17.5)	8 (18.6)	0.74
Comorbidities				
Obesity	11 (25.0)	12 (19.0)	7 (16.3)	0.34
Hypertension	24 (54.5)	36 (57.1)	25 (58.1)	0.74
Dyslipidemia	14 (31.8)	32 (50.8)	21 (48.8)	0.11
Diabetes	4 (9.1)	10 (15.9)	1 (2.3)	0.30
Sleep apnea	2 (4.5)	7 (11.1)	6 (14.0)	0.14
CPAP use	3 (6.8)	6 (9.5)	3 (7.0)	0.98
CKD	5 (11.4)	8 (12.7)	5 (11.6)	0.92
Medical history				
Cerebrovascular accident	2 (4.5)	3 (4.8)	4 (9.3)	0.35
Heart failure	1 (2.3)	7 (11.1)	5 (11.6)	0.12
Peripheral vascular disease	0 (0.0)	3 (4.8)	1 (2.3)	0.48
Ischemic cardiomyopathy	2 (4.5)	2 (3.2)	4 (9.3)	0.33
Neoplasia	4 (9.1)	3 (4.8)	2 (4.7)	0.38
Type of AF				
Paroxysmal	23 (52.3)	39 (61.9)	24 (55.8)	0.73
Persistent	21 (47.7)	24 (38.1)	19 (44.2)	
Duration of AF, months	23.0 (3.0-69.0)	24.0 (5.8-72.0)	36.0 (7.0-96.0)	0.14
EHRA score				
1	10 (22.7)	10 (15.9)	8 (18.6)	
2	27 (61.4)	38 (60.3)	22 (51.2)	0.18
3 and 4	7 (15.9)	15 (23.8)	13 (30.2)	
Computed tomography				
LA diameter, mm	59.9 ± 6.4	61.1 ± 8.1	63.1 ± 8.4	0.06
Conventional echocardiography				
LVEF, %	61.2 ± 6.8	60.3 ± 8.1	60.9 ± 9.6	0.86
LA volume index, ml/m^2	26.9 ± 9.9	32.1 ± 13.0	34.3 ± 13.9	0.016

Values are mean ± SD, n (%), or median (interquartile range). Obesity was defined as BMI >30 kg/m^2. Hypertension was defined as systolic blood pressure >139 mm Hg, diastolic blood pressure >89 mm Hg, or antihypertensive treatment. Dyslipidemia was defined by the presence of hypercholesterolemia (serum cholesterol >200 mg/dl) and/or hypertriglyceridemia (serum triglycerides >150 mg/dl). CKD was defined as eGFR <60 ml/min/1.73 m^2.

Abbreviations as in Tables 1, 2, and 4.

FIGURE 2 Recurrence of AF in Patients Referred to AF Ablation Classified According to Study Subgroups



Kaplan-Meier curves for the incidence of post-ablation AF recurrence in patients with AF classified in 3 groups according to the presence (+) or absence (–) of increased CCL and increased CD, based on the serum carboxy-terminal telopeptide of collagen type I to matrix metalloproteinase-1 ratio and the serum carboxy-terminal propeptide of procollagen type I threshold values, respectively, as defined in the main text. Abbreviations as in Figure 1.

TABLE 6 Hazard Ratio and Added Predictive Value of the Combinations of Biomarkers for Recurrence of Atrial Fibrillation in the 150 Post-Ablation Patients

Statistic Parameter	Hazard Ratio	95% CI	p Value
Unadjusted Cox regression			
CCL−CD− (reference)	1.00		
CCL−CD+ or CCL+CD−	1.58	0.65-3.88	0.32
CCL+CD+	3.85	1.64-9.03	0.002
Adjusted Cox regression*			
CCL−CD− (reference)	1.00		
CCL−CD+ or CCL+CD−	1.39	0.56-3.48	0.48
CCL+CD+	3.38	1.37-8.29	0.008
Added predictive value			
NRI	0.60	0.18-1.02	0.005

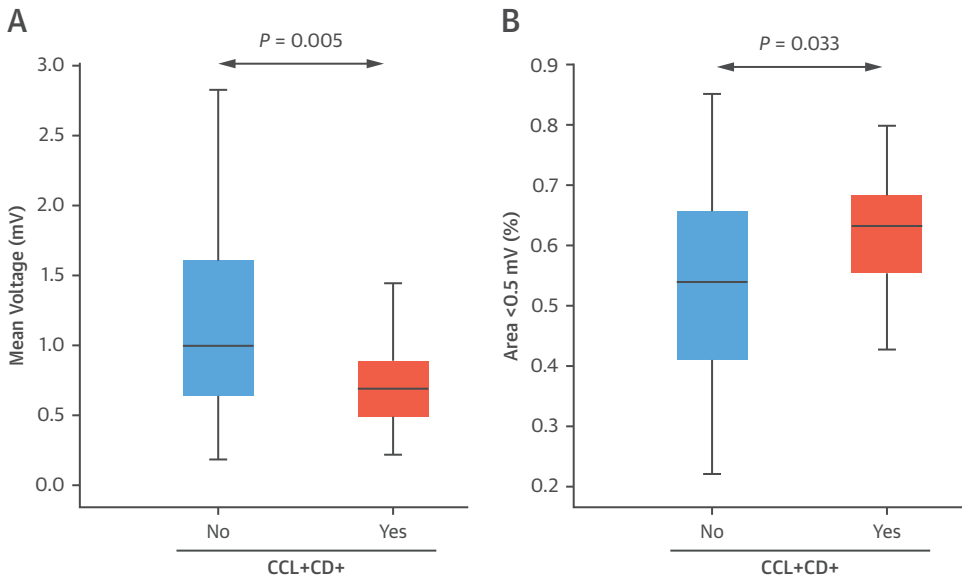
*Hazard ratios were adjusted by the basal model: age, persistent atrial fibrillation (yes/no) and left atrial volume index. Patients were classified as with (+) or without (–) increased collagen type I cross-linking (CCL) and increased collagen type I deposition (CD) considering the carboxy-terminal telopeptide of collagen type I to the matrix metalloproteinase-1 ratio and the carboxy-terminal propeptide of procollagen type I thresholds, respectively, as defined in the main text.

NRI = net reclassification improvement.

that was accompanied by increased collagen cross-linking and content (13). The composition of collagen fibers (i.e., collagen type I vs. collagen type III) also influences the properties of collagen deposits, as human collagen type I fibers are stiffer than collagen type III fibers (25). Of interest, collagen type I fiber content and atrial stiffness are increased in AF patients compared with patients in sinus rhythm (13,26). Finally, the amount of collagen type I fibers is regulated by both the production of procollagen type I by myofibroblasts and the extracellular processing of procollagen type I into fibril-forming collagen type I molecules by specific proteinases, such as the bone morphogenetic protein-1 that removes PICP, which is further released into the blood stream (9). Of notice, serum PICP has been found to correlate with the presence of LA fibrosis in patients without AF submitted to cardiac surgery (20) and in AF patients (21).

The observation that the risk of AF recurrence is particularly high in AF patients with the CCL+CD+

FIGURE 3 Distribution of V_m and of the Percentage of the Left Atrial Area With V_m Values <0.5 mV in Patients Referred for AF Ablation Classified According to Presence or Absence of the CCL+CD+ Combination of Biomarkers



Box plots show the 5th and 95th **(vertical lines)**, 25th and 75th **(boxes)**, and 50th **(horizontal line)** percentile values for the V_m **(A)** and the percentage of the left atrial area with V_m values <0.5 mV **(B)** in patients with AF classified according to the absence or presence of increased collagen type I cross-linking and increased collagen type I deposition (CCL+CD+) based on the serum carboxy-terminal telopeptide of collagen type I to matrix metalloproteinase-1 ratio and the serum carboxy-terminal propeptide of procollagen type I threshold values, respectively, as defined in the main text. V_m = mean atrial bipolar voltage; other abbreviations as in Figure 1.

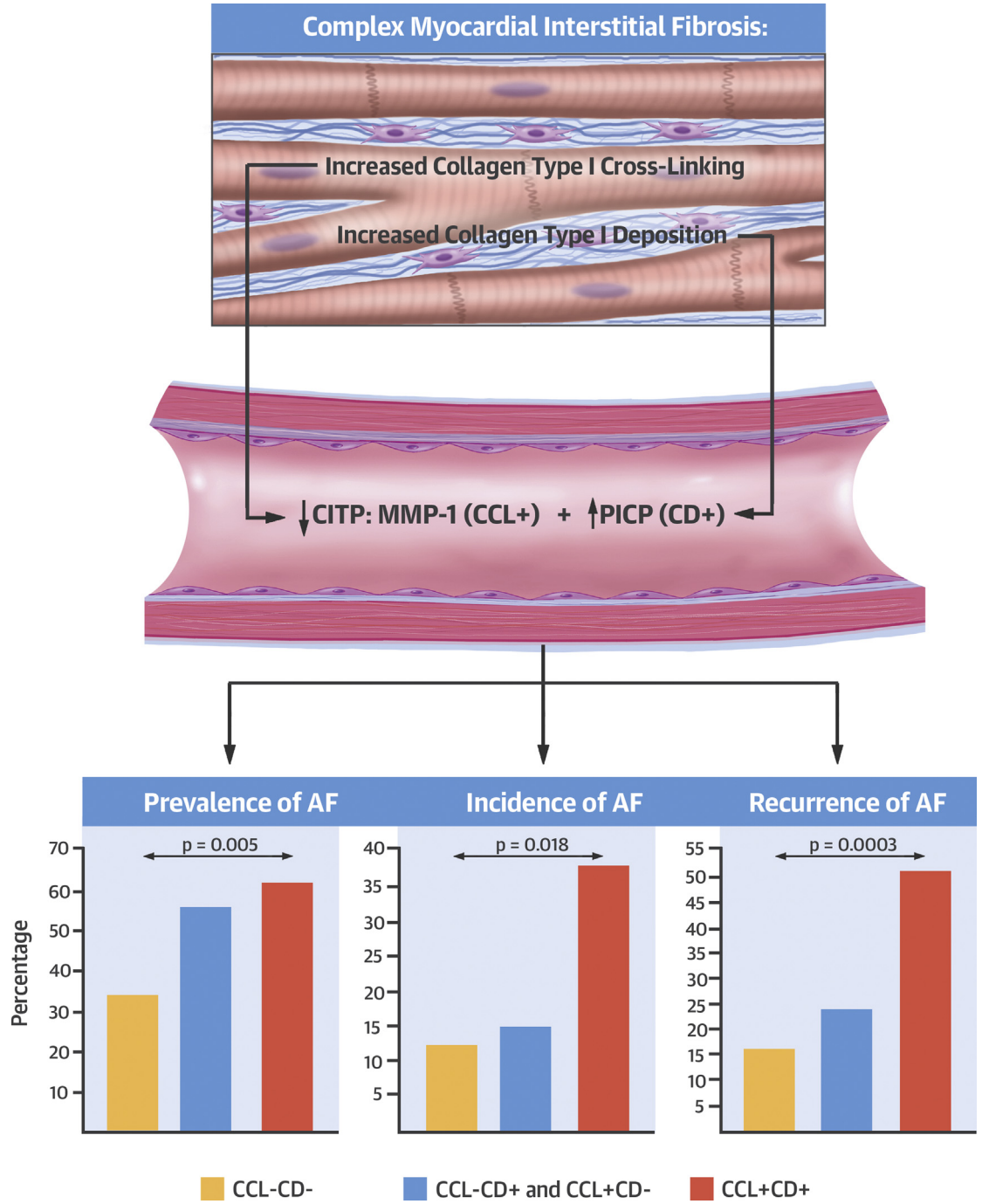
combination of biomarkers suggests that the efficacy of the AF ablation procedure is not optimal in patients with a high myocardial deposition of highly cross-linked collagen type I fibers, opening up the possibility of utilizing this combination of biomarkers to consider the convenience of PV ablation in these patients. From this perspective, it is tempting to speculate that additional therapies with proven efficacy to reduce both myocardial collagen type I cross-linking and deposition in cardiac patients (e.g., torasemide [27,28]) and animals (e.g. statins [13] and losartan [29]) might be of additional benefit for AF patients with the CCL+CD+ combination of biomarkers.

STUDY LIMITATIONS. First, the study in HF patients was retrospective and the number of patients enrolled was limited, especially when assessing new-onset AF. On the other hand, study 2, which was prospectively designed in a different population, showed compatible results, reinforcing the validity of the findings. Even so, the findings on the diagnostic and prognostic usefulness of the CCL+CD+ biomarker combination are hypothesis generating. Second, external validation in larger and independent cohorts is necessary. Finally, the associations found between the CCL+CD+ biomarker combination and the assessed clinical outcomes do not necessarily establish causality, and the association with low voltage atrial areas do not inexorably means that the origin of the biomarkers is the heart.

CONCLUSIONS

The CCL+CD+ combination of circulating biomarkers is associated with the prevalence and the incidence of AF in HF patients, suggesting that a complex type of MIF characterized by both increased collagen type I cross-linking and increased collagen type I deposition determines the risk of AF in HF patients (Central Illustration). In addition, the CCL+CD+ combination of biomarkers is associated with larger left atrium low-voltage areas and

CENTRAL ILLUSTRATION **Atrial Fibrillation and Biomarkers of Myocardial Fibrosis**



Ravassa, S. et al. J Am Coll Cardiol. 2019;73(12):1398–410.

Identification of a combination of collagen type I-related circulating biomarkers with prognostic utility in atrial fibrillation (AF). The development of a pattern of complex myocardial interstitial fibrosis (MIF) characterized by increased collagen type I cross-linking (CCL+) and increased collagen type I deposition (CD+) can be crucial in the development and maintenance of the arrhythmogenic substrate of atrial fibrillation (AF). These 2 alterations of collagen metabolism can be detected noninvasively by the assessment of 2 circulating biomarkers: a low ratio of serum carboxy-terminal telopeptide of collagen type I to serum matrix metalloproteinase-1 (↓ CITP:MMP-1) that reflects CCL+, and a high serum carboxy-terminal propeptide of procollagen type I (↑ PICP) level that reflects CD+. Patients with the CCL+CD+ combination of biomarkers present a higher risk of AF prevalence, new-onset AF, and AF recurrence after ablation than patients without this combination of biomarkers.

identifies those patients who are at high risk of AF recurrence after ablation, suggesting that the same particular type of MIF previously mentioned is responsible for maintaining an arrhythmogenic substrate refractory to the catheter ablation procedure in AF patients (**Central Illustration**).

ADDRESS FOR CORRESPONDENCE: Dr. Javier Díez, Program of Cardiovascular Diseases, Center for Applied Medical Research, Avenida Pío XII 55, 31008 Pamplona, Spain. E-mail: jadimar@unav.es. OR Dr. Ignacio García-Bolao, Department of Cardiology and Cardiac Surgery, University of Navarra Clinic, Avda. Pío XII 36, 31008 Pamplona, Navarra, Spain. E-mail: igarciab@unav.es. Twitter: @ClinicaNavarra.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: A combination of circulating biomarkers of increased myocardial collagen type I cross-linking and deposition (i.e., CCL+CD+) may identify patients who are at high risk of developing AF and/or its recurrence after ablation.

TRANSLATIONAL OUTLOOK: Further studies are required to establish whether specific therapeutic strategies to correct myocardial collagen metabolism in patients with the CCL+CD+ biomarkers reduce the risk of developing de novo AF or its recurrence after ablation.

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APPENDIX For an expanded Methods section as well as supplemental tables and figures, please see the online version of this paper.