# Relation of Peripheral Collagen Markers to Death and Hospitalization in Patients With Heart Failure and Preserved Ejection Fraction

## Results of the I-PRESERVE Collagen Substudy

Henry Krum, MBBS, PhD, FRACP; Maros Elsik, MBBS, PhD, FRACP; Hans G. Schneider, MBBS, PhD, FRACP; Agata Ptaszynska, MD; Marion Black, MSc; Peter E. Carson, MD; Michel Komajda, MD; Barry M. Massie, MD; Robert S. McKelvie, MD; John J. McMurray, MBChB; Michael R. Zile, MD; Inder S. Anand, MD, FRCP, DPhil (Oxon)

***Background***—Heart failure with preserved ejection fraction (HFPEF) is a common and increasing public health problem. Myocardial fibrosis is a key pathological feature of HFPEF. Peripheral collagen markers may reflect this excess fibrosis; however, the relation of these markers to prognosis in patients with HFPEF has not as yet been determined.

***Methods and Results***—This substudy of the Irbesartan in Heart Failure With Preserved Systolic Function (I-PRESERVE) trial measured plasma levels of procollagen type I amino-terminal peptide, procollagen type III amino-terminal peptide, and osteopontin in 334 patients with HFPEF. Measurements were performed at baseline and 6 months after randomization to placebo or irbesartan 300 mg/day. The relation of baseline collagen markers to the I-PRESERVE primary end point (all-cause death and hospitalization for prespecified cardiovascular causes) was evaluated by single and multivariable analysis. Similar evaluations were performed for all-cause death alone as well as heart failure events (death or hospitalization because of heart failure). Increased plasma levels of collagen markers at baseline were associated with increased frequency of the study primary end point for all collagen markers. For each 10-$\mu$g/L increase in procollagen type I amino-terminal peptide, the hazard ratio (HR) for the primary end point was 1.09 (95% CI, 1.052 to 1.13; $P<0.0001$); for each 10-$\mu$g/L increase in procollagen type III amino-terminal peptide procollagen type I amino-terminal peptide, the HR was 2.47 (95% CI, 0.97 to 6.33; $P=0.059$); and for each 10-nmol/L increase in osteopontin, the HR was 1.084 (95% CI, 1.026 to 1.15; $P=0.004$). No variable remained significant as an independent predictor when introduced into a multivariable model. Both treatment groups tended to reduce collagen markers, with the reduction significantly greater for placebo versus irbesartan for procollagen type III amino-terminal peptide only ($P=0.0185$).

***Conclusions***—Increased peripheral collagen turnover markers were not independently associated with increased mortality and cardiovascular hospitalization in an HFPEF population on multivariable analysis but were associated on single-variable analysis. These findings provide some support to the hypothesis that pathological fibrosis in the heart, and possibly the peripheral vasculature, may be contributory to adverse clinical outcomes in patients with HFPEF.

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**Key Words:** heart failure ■ diastolic pressure ■ ventricular ejection fraction ■ collagen ■ fibrosis ■ osteopontin



Heart failure with preserved ejection fraction (HFPEF) is a common and increasing public health problem.[1] Its prevalence appears to be similar to that of patients with heart failure with reduced ejection fraction (HFREF). Furthermore, long-term major clinical outcomes associated with HFPEF are poor, although in general, they are slightly better than for HFREF.[2]

**Clinical Perspective on p 568**

Unlike HFREF for which there are a number of known therapeutic strategies available to ameliorate morbidity and mortality, there is no therapy that has been demonstrated to be beneficial in the management of patients with HFPEF. This may relate in part to an incomplete understanding of the

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From the Department of Epidemiology and Preventive Medicine, Centre of Cardiovascular Research and Education in Therapeutics, Monash University/Alfred Hospital (H.K., M.E.), and Pathology Department, Alfred Hospital (H.G.S., M.B.), Melbourne, Victoria, Australia; Bristol-Myers Squibb, Princeton, NJ (A.P.); Georgetown University, Washington, DC (P.E.C.); Universite Paris 6, Paris, France (M.K.); University of California, San Francisco, and the San Francisco VA Medical Center, San Francisco, CA (B.M.M.); Hamilton Health Sciences, McMaster University, Hamilton, Ontario, Canada (R.S.M.); University of Glasgow, Glasgow, UK (J.J.M.); RHJ Department, VA Medical Center, and Medical University of South Carolina, Charleston, SC (M.R.Z.); and VA Medical Centre and University of Minnesota, Minneapolis, MN (I.S.A.).

Correspondence to Henry Krum, MBBS, PhD, FRACP, Department of Epidemiology and Preventive Medicine, Centre of Cardiovascular Research and Education in Therapeutics, Monash University/Alfred Hospital, Melbourne, Victoria 3004, Australia. E-mail henry.krum@monash.edu



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pathophysiology that underlies this condition. However, factors that appear to be contributory to this pathophysiology include hypertension, coronary ischemia, and the cardiac sequelae of diabetes mellitus (if present).[1] A common pathophysiological accompaniment of HFPEF associated with all these underlying etiologies is the accumulation of extracellular matrix (ECM) material[3] within the heart (myocardial fibrosis). ECM accumulation is, in turn, believed to be a major contributor to the impaired cardiac relaxation that is the hallmark of this condition.[4]

Clinical evaluation of the presence and magnitude of ECM accumulation in the heart is difficult. The gold standard for such evaluation is myocardial biopsy, but even then, fibrosis may be missed if it is patchy and nonhomogeneously distributed through the heart.[5] Various imaging modalities have been proposed[5] in an attempt to quantitate pathological ECM distribution, but these may suffer from methodological deficiencies in identifying true pathological fibrosis.

The use of biochemical markers also have been attempted in this regard. Peripheral markers of collagen turnover have been studied extensively in conditions such as hypertension, left ventricular hypertrophy, and HFREF.[6–8] However, there has been little evaluation[9] of peripheral collagen markers in patients with HFPEF despite the aforementioned importance of this pathophysiological biomarker. In particular, the utility of such markers in predicting major cardiovascular and mortal events has as yet not been undertaken.

We therefore conducted a substudy within the Irbesartan in Heart Failure With Preserved Systolic Function (I-PRESERVE) trial[10] in which patients with HFPEF were randomized to the angiotensin receptor blocker irbesartan or to placebo to evaluate the impact of an angiotensin receptor blocker on peripheral collagen markers as well as the clinical utility of these markers in predicting such events.

In this prespecified substudy, we hypothesized that (1) irbesartan (through blockade of the profibrotic peptide angiotensin II) would reduce pathological fibrosis as reflected by reductions in peripheral collagen turnover markers compared with placebo and (2) plasma levels of ≥1 peripheral collagen marker measured at baseline would be predictive of future major cardiovascular events in an HFPEF population.

# Methods

## Patient Population

A subgroup of sites involved in the overall I-PRESERVE trial[10] were invited to contribute patients to the I-PRESERVE peripheral collagen marker substudy; 334 patients with HFPEF subsequently were recruited into this substudy. Of these, 313 patients had full baseline and 6-month assessments.

## Laboratory Evaluation

We evaluated 3 clinically relevant peripheral markers of collagen turnover: procollagen type I amino-terminal peptide (PINP), procollagen type III amino-terminal peptide (PIIINP), and osteopontin. Regarding preanalytical handling of blood samples, blood was spun at 1800 rpm in a cold (4°C) centrifuge and plasma was extracted and stored frozen at −20°C for transfer to the laboratory on dry ice. PINP was measured by electroluminescence assay on the Cobas E170 analyzer (Roche Diagnostics, Castel Hill, NSW, Australia), PIIINP was measured by radioimmunoassay (Orion Diagnostica; Espoo, Finland), and osteopontin by enzyme-linked immunosorbent assay (IBL International; Hamburg, Germany). Intraassay and interassay coefficients of variability were <10% for all 3 biomarkers.

## Study Design

Plasma levels of PINP, PIIINP, and osteopontin were measured at baseline and 6 months. Baseline measures were used to determine the utility of such peripheral collagen markers in predicting major events. These events comprised the main study's primary end point of being a composite of all-cause mortality and cardiovascular hospitalization (time to first event), all-cause mortality alone, and a heart failure composite comprising death of heart failure and hospitalization for heart failure. All clinical events were evaluated at the end of the study. Changes in collagen markers between baseline and 6 months were used to evaluate the impact of irbesartan versus placebo on levels of such markers.

## Statistical Analysis and Sample Size

The sample size for this substudy was based on the RALES (Randomized Aldactone Evaluation Study) collagen substudy, assuming that similar findings would be observed in this HFPEF population with irbesartan versus placebo as with spironolactone versus placebo in RALES.[8] In the RALES study of advanced HFREF, a significant reduction in plasma collagen markers was observed with the aldosterone antagonist spironolactone versus placebo in a sample of 261 patients.[8] With an SD of 6% in measurement of PINP, clinically relevant difference of 20% $\alpha$=0.05, and power of 80%, a minimum of 120 patients were required in each group to allow for dropouts. A minimum of 300 patients were, therefore, recruited.

Multiple linear regression analysis was performed to evaluate which baseline variables were independently associated with baseline plasma collagen markers. The following variables were included in the analysis: age, sex, New York Heart Association functional class, systolic blood pressure, hemoglobin level, estimated glomerular filtration rate, left ventricular ejection fraction, ischemic etiology, hypertension etiology, history of atrial fibrillation, history of diabetes, history of hospitalization in the previous 6 months, albumin level, neutrophils, pulmonary congestion on chest radiograph, history of chronic obstructive pulmonary disease, heart rate, body mass index, and serum sodium. Cox proportional hazards regression models were used to assess the association between baseline plasma collagen markers and time to the primary end point and the 2 secondary end points of time to all-cause mortality and time to the heart failure composite end point. PINP was evaluated per 10-$\mu$g/L increase, PIIINP per 10-$\mu$g/L increase, and osteopontin per 10-nmol/L increase. Three models were evaluated for each end point. First, each marker was tested alone in a univariable analysis. A multivariable model then was tested that included the aforementioned 19 variables (model 1). Model 2 was model 1 plus plasma level of N-terminal pro-brain natriuretic peptide. The planned sample size for this substudy based on the first hypothesis to be tested (effect of irbesartan versus placebo on change in collagen biomarker levels) is likely underpowered in the evaluation of the second hypothesis to be tested (relation of baseline plasma levels of peripheral collagen markers to future major cardiovascular events).

# Results

## Study Patients

Baseline characteristics of the patients enrolled in the main I-PRESERVE study according to treatment received as well as the collagen substudy population of 313 patients are summarized in Table 1. Mean exposure to therapy in both the main I-PRESERVE study and the collagen substudy was similar at 49.5 months.

**Table 1. Baseline Characteristics of Patients in the Entire Cohort and Collagen Substudy by Treatment**

| | Entire Study | | Collagen Substudy | |
|---|---|---|---|---|
| Characteristic | Placebo (n=2061) | Irbesartan (n=2067) | Placebo (n=164) | Irbesartan (n=149) |
| Demographic | | | | |
| Age, y | 72±7 | 72±7 | 72±7 | 72±7 |
| Age ≥75 y | 716 (35) | 697 (34) | 63 (37) | 61 (36) |
| Female sex | 1264 (61) | 1227 (59) | 106 (62) | 101 (60) |
| Race | | | | |
| White | 1925 (93) | 1934 (94) | 153 (93) | 136 (91) |
| Nonwhite | 136 (7) | 133 (6) | 12 (7) | 16 (10) |
| Clinical | | | | |
| NYHA class | | | | |
| II | 444 (22) | 426 (21) | 39 (23) | 40 (24) |
| III | 1562 (76) | 1582 (77) | 131 (77) | 126 (75) |
| IV | 53 (3) | 59 (3) | 1 (0.6) | 2 (1.2) |
| Systolic BP, mm Hg | 136±15 | 137±15 | 136±15 | 136±17 |
| Diastolic BP, mm Hg | 79±9 | 79±9 | 79±9 | 78±10 |
| Heart rate, beats/min | 71±10 | 72±11 | 71±10 | 71±10 |
| Hemoglobin, g/dL | 13.9±1.9 | 13.9±13.9 | 13.9±1.9 | 13.8±1.9 |
| eGFR, mL/min per 1.73 $m^2$ | 72±22 | 73±23 | 75±25 | 73±24 |
| Serum sodium, mmol/L | 140±3 | 140±3 | 139±3 | 139±2 |
| Ejection fraction, % | 60±9 | 59±9 | 61±9 | 59±10 |
| Cause of HF | | | | |
| Ischemia | 500 (24) | 536 (26) | 40 (23) | 41 (24) |
| Hypertension | 1304 (63) | 1318 (64) | 119 (70) | 105 (63) |
| Hospitalization for HF* | 906 (44) | 910 (44) | 56 (32) | 56 (33) |
| Medical history | | | | |
| Hypertension | 1816 (88) | 1834 (89) | 156 (91) | 145 (86) |
| Myocardial infarction | 482 (23) | 487 (24) | 33 (19) | 33 (20) |
| PCI or CABG | 267 (13) | 281 (14) | 25 (15) | 35 (21) |
| Atrial fibrillation | 603 (29) | 606 (29) | 49 (29) | 49 (29) |
| Diabetes mellitus | 564 (27) | 570 (28) | 59 (35) | 48 (29) |
| Stroke or TIA | 201 (10) | 198 (10) | 20 (12) | 17 (10) |
| Quality of life | 42±20 | 42±21 | 39±19 | 38±21 |
| Median NT-proBNP, pg/mL | 320 (131–946) | 360 (139–987) | 382 (131–1096) | 392 (115–978) |
| Medication | | | | |
| Diuretic | 1675 (84) | 1648 (83) | 130 (77) | 124 (77) |
| Loop | 1041 (52) | 1038 (52) | 67 (39) | 75 (46) |
| Thiazide | 779 (38) | 776 (38) | 55 (32) | 48 (30) |
| Spironolactone | 309 (16) | 312 (16) | 24 (14) | 24 (15) |
| ACE inhibitor | 510 (25) | 538 (26) | 68 (40) | 51 (32) |
| Digoxin | 269 (13) | 291 (14) | 12 (7) | 23 (14) |
| $\beta$-blocker | 1169 (59) | 1191 (60) | 90 (53) | 91 (56) |
| Calcium-channel blocker | 787 (39) | 802 (40) | 70 (41) | 56 (35) |
| Nitrate | 534 (27) | 539 (27) | 27 (16) | 29 (18) |

Data are presented as mean±SD, n (%), or median (interquartile range). ACE indicates angiotensin-converting enzyme; BP, blood pressure; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; HF, heart failure; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

*Within previous 6 months.

**Table 2. Primary End Point of All-Cause Mortality and HF Composite Event in Relation to Baseline OPN Levels**

| | No. Patients | No. Events | % Events | HR | 95% CI | *P* |
|---|---|---|---|---|---|---|
| Primary end point | | | | | | |
| OPN alone (per 10 nmol/L) | 317 | 94 | 29.6 | 1.08 | 1.03–1.15 | 0.004 |
| Model 1 plus OPN | 274 | 81 | 29.5 | 0.98 | 0.92–1.06 | 0.653 |
| Model 2 plus OPN | 246 | 75 | 30.5 | 0.97 | 0.90–1.05 | 0.45 |
| All-cause mortality | | | | | | |
| OPN alone (per 10 nmol/L) | 317 | 49 | 15.4 | 1.06 | 1.02–1.11 | 0.007 |
| Model 1 plus OPN | 274 | 42 | 15.3 | 1.02 | 0.92–1.13 | 0.71 |
| Model 2 plus OPN | 246 | 39 | 15.8 | 0.99 | 0.88–1.11 | 0.823 |
| HF composite events | | | | | | |
| OPN alone (per 10 nmol/L) | 317 | 42 | 13.3 | 1.06 | 0.99–1.14 | 0.083 |
| Model 1 plus OPN | 274 | 37 | 13.5 | 0.99 | 0.89–1.09 | 0.839 |
| Model 2 plus OPN | 246 | 34 | 13.8 | 0.93 | 0.83–1.05 | 0.244 |

HR, hazard ratio; OPN, osteopontin. Other abbreviation as in Table 1.

## Baseline Collagen Marker Levels and Clinical Correlates

The median plasma level of PINP at baseline was 43.8 μg/L (Q1 to Q3, 28.9 to 63.5 μg/L; minimum to maximum, 7.1 to 350 μg/L; n=333). The reference range for plasma PINP is premenopausal women, <59 μg/L; postmenopausal women, <76 μg/L; men aged 25 to 70 years, 15 to 80 μg/L; and men aged >70 years, >80 μg/L. Baseline variables independently associated with baseline plasma PINP were baseline estimated glomerular filtration rate, history of heart failure, and history of atrial fibrillation.

The median plasma level of PIIINP at baseline was 4.3 μg/L (Q1 to Q3, 3.68 to 5.4 μg/L; minimum to maximum, 1 to 20 μg/L; n=334). The reference range for plasma PIIINP is 2.3 to 6.4 μg/L in adults aged 19 to 65 years. Baseline variables independently associated with baseline plasma PINP were age, baseline estimated glomerular filtration rate, baseline body mass index, and history of diabetes mellitus.

The median plasma level of osteopontin was 23 nmol/L (Q1 to Q3, 10.14 to 47.4 nmol/L; minimum to maximum, 5 to 262 nmol/L; n=317). No reference range for plasma osteopontin has as yet been established. Baseline variables independently associated with baseline plasma PINP were male sex, age, baseline estimated glomerular filtration rate, ischemic etiology, history of atrial fibrillation, and etiology of hypertension. All baseline collagen markers were strongly positively correlated with baseline plasma N-terminal pro-brain natriuretic peptide levels ($r$=0.392 to 0.616, $P$<0.001 to 0.008).

## Relation of Collagen Markers to Major Cardiovascular Events

### *Baseline Plasma Collagen Marker Levels*

Increased plasma levels of collagen markers at baseline were associated with increased frequency of key study end points on single variable analysis (Tables 2 through 4). For baseline osteopontin levels, there was a significant association with the primary I-PRESERVE end point of all-cause mortality and a borderline association with heart failure composite

**Table 3. Primary End Point of All-Cause Mortality and HF Composite Event in Relation to Baseline PINP Levels**

| | No. Patients | No. Events | % Events | HR | 95% CI | *P* |
|---|---|---|---|---|---|---|
| Primary end point | | | | | | |
| PINP alone 10 μg/L | 333 | 101 | 30.3 | 1.09 | 1.05–1.13 | <0.0001 |
| Model 1 plus PINP | 287 | 85 | 29.6 | 1.001 | 0.96–1.05 | 0.98 |
| Model 2 plus PINP | 257 | 78 | 30.3 | 0.99 | 0.95–1.04 | 0.637 |
| All-cause mortality | | | | | | |
| PINP alone 10 μg/L | 333 | 52 | 15.6 | 1.06 | 1.03–1.09 | <0.001 |
| Model 1 plus PINP | 287 | 44 | 15.3 | 1.04 | 0.98–1.10 | 0.24 |
| Model 2 plus PINP | 257 | 40 | 15.5 | 1.03 | 0.96–1.10 | 0.43 |
| HF composite events | | | | | | |
| PINP alone 10 μg/L | 333 | 46 | 13.8 | 1.09 | 1.05–1.13 | <0.0001 |
| Model 1 plus PINP | 287 | 39 | 13.5 | 1.03 | 0.97–1.1 | 0.275 |
| Model 2 plus PINP | 257 | 36 | 14 | 1.02 | 0.96–1.09 | 0.526 |

PINP indicates procollagen type I amino-terminal peptide. Other abbreviations as in Tables 1 and 2.

**Table 4. Primary End Point of All-Cause Mortality and HF Composite Event in Relation to Baseline PIIINP Levels**

| | No. Patients | No. Events | % Events | HR | 95% CI | *P* |
|---|---|---|---|---|---|---|
| Primary end point | | | | | | |
| PIIINP alone 10 μg/L | 334 | 102 | 30.5 | 2.47 | 0.97–6.33 | 0.059 |
| Model 1 plus PIIINP | 287 | 86 | 29.9 | 1.06 | 0.37–3.02 | 0.912 |
| Model 2 plus PIIINP | 259 | 80 | 30.8 | 0.84 | 0.27–2.62 | 0.764 |
| All-cause mortality | | | | | | |
| PIIINP alone 10 μg/L | 334 | 52 | 15.6 | 2.85 | 1.52–5.36 | 0.001 |
| Model 1 plus PIIINP | 287 | 44 | 15.3 | 0.99 | 0.193–5.14 | 0.998 |
| Model 2 plus PIIINP | 259 | 40 | 15.4 | 0.80 | 0.13–4.92 | 0.813 |
| HF composite events | | | | | | |
| P3NP alone 10 μg/L | 334 | 48 | 16.8 | 5.91 | 2.94–11.88 | 0.0001 |
| Model 1 plus PIIINP | 287 | 41 | 14.2 | 2.67 | 0.69–10.32 | 0.156 |
| Model 2 plus PIIINP | 259 | 38 | 14.6 | 1.70 | 0.39–7.42 | 0.479 |

PIIINP indicates procollagen type III amino-terminal peptide. Other abbreviations as in Tables 1 and 2.



events. For PINP baseline levels, all major end points were highly significant. For baseline PIIINP, the primary end point was borderline significant, whereas all-cause mortality and heart failure composite events were highly significant. In contrast, none of these markers remained significant predictors of future events when entered into the 2 multivariable models.

### *Change in Collagen Marker Levels*

Change in collagen marker over the 6 months of the study and its relation to subsequent cardiac events and death were nonsignificant overall. One exception was the change in osteopontin ($P=0.019$), which was predictive for all-cause mortality.

Kaplan–Meier plots of collagen markers (split along the median) in relation to the primary study end point (all-cause death and cardiovascular hospitalization) are shown in Figures 1 through 3 (PINP, PIIINP, and osteopontin, respectively).

### Relation of Treatment to Collagen Marker Levels

After 6 months, treatment collagen markers tended to fall in both the placebo and irbesartan groups (PINP, −3.2±1.9 versus −0.7±2.0 μg/L, *P* not significant; PIIINP, −0.4±0.1 versus −0.1±0.1 μg/L, $P=0.0185$; osteopontin, −5.6±2.1 versus −3.6±2.2 ng/mL, *P* not significant).

## Discussion

The present analyses demonstrate that peripheral collagen markers, when measured at baseline, are predictive of major cardiovascular events in an HFPEF population. Specifically, we observed that when baseline markers were split along the median (irrespective of subsequent treatment received), there was a significant increase in both time to all-cause death or adjudicated protocol-specified cardiovascular hospitalization (the main I-PRESERVE study primary end point), all-cause death alone, and the heart failure composite end point on single-variable analysis. These findings do not remain signif-

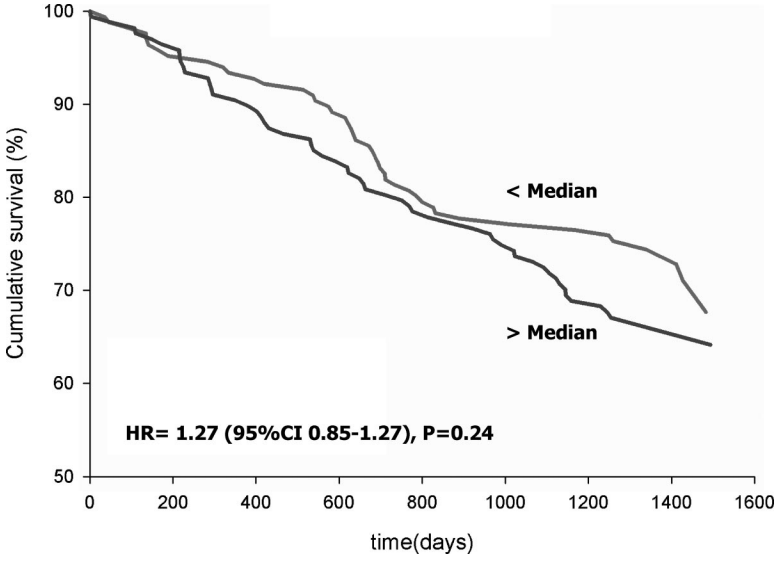


**Figure 1.** Kaplan–Meier plot by baseline procollagen type I amino-terminal peptide for time to all-cause death or adjudicated protocol-specified cardiovascular hospitalization. HR indicates hazard ratio.

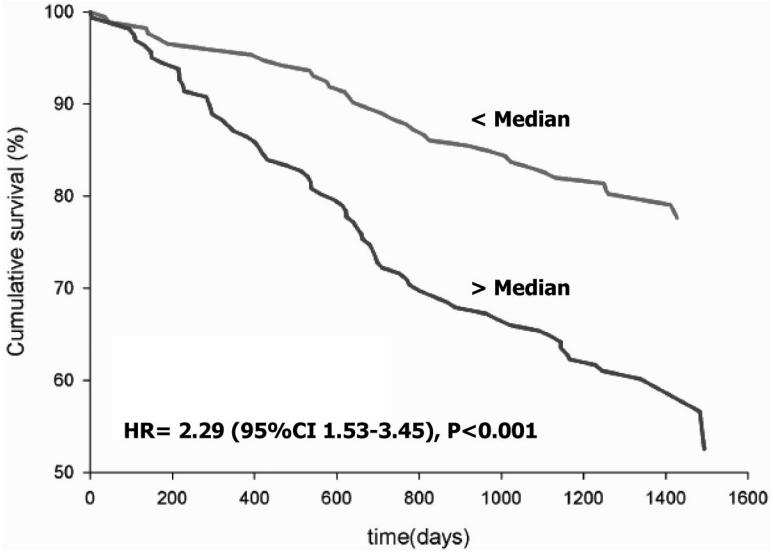


**Figure 2.** Kaplan–Meier plot by baseline procollagen type III amino-terminal peptide for time to all-cause death or adjudicated protocol-specified cardiovascular hospitalization. Abbreviation as in Figure 1.



icant, however, after adjustment for relevant baseline covariates in a multivariable model. The present findings, therefore, potentially support ECM accumulation (fibrosis) as being pathophysiologically important in the progression of the HFPEF disease process as well as being a contributor to subsequent events.

These findings also somewhat mirror observations made in patients with HFREF. Specifically, the RALES study of patients with advanced HFREF observed that PIIINP was found to be predictive of subsequent mortality in those patients.[8] In addition to traditional fibrosis markers such as the amino-terminal peptides, which have been explored extensively in HFREF and post-myocardial infarction left ventricular systolic dysfunction settings, osteopontin was assessed because it integrates both the inflammatory and the fibrotic aspects of the cardiac remodeling process and appears to be responsive to therapeutic interventions that meaningfully affect the cardiac remodeling process.[11,12]

Although plasma levels of collagen markers generally were lowered by 6 months of irbesartan therapy, these reductions were not large, generally not different from placebo, and certainly not clinically significant. These findings are in keeping with the overall I-PRESERVE study findings[13] where irbesartan therapy did not affect either the primary end point (all-cause mortality or cardiovascular hospitalization) or the all-cause mortality alone end point. The absence of impact of an angiotensin II $AT_1$ receptor antagonist on fibrosis markers is surprising because angiotensin II is a well-established profibrotic stimulus in various experimental settings, such as cardiac fibroblast cell culture.[14] One possibility is that the follow-up fibrotic marker assessment was not of sufficient duration to meaningfully lower plasma levels. For example, in the study of Mak et al,[15] PIIINP levels were lowered versus placebo at 12 months but not at 6 months.

Despite the limited nature of the relationships observed, it may be speculated from the present findings that therapies that beneficially affect pathological ECM deposition in patients with HFPEF may reduce major events in this population. This is of considerable relevance because there have been as yet no therapies proven to be of benefit in this

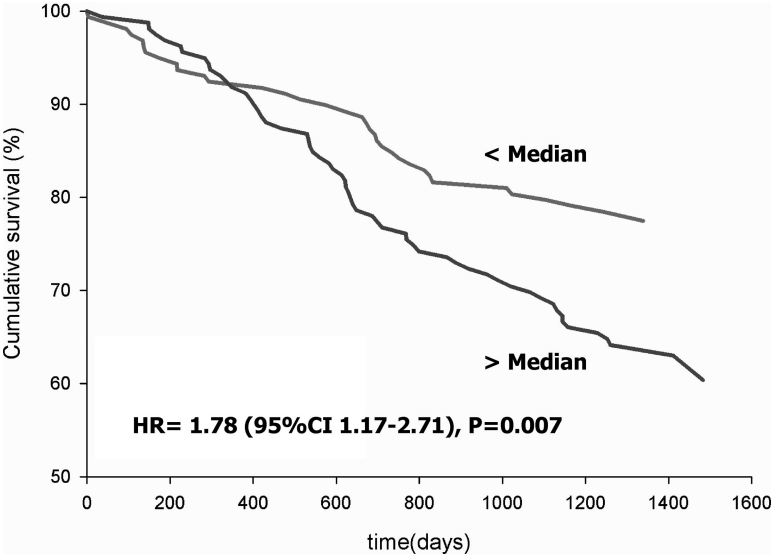


**Figure 3.** Kaplan–Meier plot by baseline osteopontin for time to all-cause death or adjudicated protocol-specified cardiovascular hospitalization. Abbreviation as in Figure 1.

condition. A number of direct antifibrotic pharmacological agents have been developed; the present findings in an HFPEF population suggest that this condition may be a useful therapeutic target for such treatments. Preclinical studies with agents such as FT-11 have demonstrated improvements in a diabetic model of HFPEF (Ren-2, streptozotocin).[16]

To our knowledge, this study is the first published analysis of the relationship between peripheral collagen markers and major cardiovascular outcomes in patients with HFPEF. A study by Rossi et al[17] looked at the relationship of collagen turnover markers, echocardiographic features of diastolic dysfunction, and survival in a cohort of 106 patients. However, unlike the present study of carefully delineated patients with HFPEF, the Rossi study patients had mean ejection fractions of 25% to 36% across the groups studied.

There are a number of caveats to the present study. The first is that it is a relatively small subpopulation of the overall I-PRESERVE study. However, patients appear to be extremely well matched in comparison with both the entire study group and the placebo and irbesartan arms of the substudy itself. Next, there are a number of organ-specific contributors to plasma levels of these collagen markers, not just the heart. In particular, bone[18] and liver[19] also are important contributors, thus potentially confounding the results of this analysis. However, we did not specifically exclude patients with liver and bone disorders because we wanted to test the utility of these markers clinically in an HFPEF population, irrespective of influencing comorbidities. Finally, the collagen markers measured were not independently predictive of future events by multivariable analysis, limiting the interpretability of the results obtained. Indeed, in some (but not all) cases, the HR for future events reverses on multivariable analysis. Moreover, the inclusion of large number of variables in the multivariable analysis for a relatively small number of events could have led to overfitting of the model. Hence, the results of the multivariable analyses, especially for the secondary end points should be interpreted with caution.

Despite these caveats, elevated baseline collagen markers were found to be associated with future major cardiovascular events in an HFPEF population on univariable (but not multivariable) analysis. Overall, these findings provide some support to hypothesis that pathological fibrosis in the heart, and possibly the peripheral vasculature, is contributory to adverse clinical outcomes in patients with HFPEF.

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## Disclosures

Dr Krum has received honoraria for being the I-PRESERVE Australia coordinator. Dr Schneider has received research grant support for NGAL in congestive heart failure, fatty acid-binding protein in brain injury, and high-sensitivity troponin in general medicine; received other research support from Janssen-Cilag for brain natriuretic peptide study; received expense payment only from Abbott and Roche; has served as an expert witness for troponin in diagnosis of acute myocardial infarction (legal case); and has served as an expert witness for Victorian Ombudsman. Dr Ptaszynska is an employee of Bristol-Myers Squibb, who sponsored data collection. Dr Michel Komajda has served on the speakers bureau for Servier, Menarini, Bristol-Meyers Squibb, AstraZeneca, GlaxoSmithKline, and Sanofi-Aventis and has received honoraria from Servier, Duke University, Nile Therapeutics, and Sanofi-Aventis. Dr Massie has received research grant support from a Veterans Administration (VA) nonprofit foundation and received payments to cover the costs for patients enrolled at the site, has received other research support from a VA research grant related to implantable cardioverter-defibrillator in the VA system, and has received consultant fees from Bristol-Meyers Squibb for time spent as principal investigator of the trial. Dr McKelvie has received honoraria from Bristol-Meyers Squibb. Dr McMurray has received research support from Glasgow University for participation as co-principal investigator in a clinical trial program that included a study in HFPEF. Dr Zile serves as a member of the Executive Committee and chairman of the EndPoints Committee. Dr Anand has received research grant support as principal investigator on I-Preserve and honoraria as a member of the EndPoint Committee. Drs Krum, Carson, Komajda, Massie, McKelvie, McMurrray, Zile, and Anand are members of the I-PRESERVE Steering Committee and have received honoraria and travel support from Bristol-Myers Squibb to attend Steering Committee meetings.

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## CLINICAL PERSPECTIVE

Heart failure with preserved ejection fraction (HFPEF) still is a poorly understood condition with no proven evidence-based effective therapy despite its large public health impact. Understanding the pathophysiology of this condition, therefore, is critical to develop specifically focused treatment strategies. The findings of the present study indicate that plasma levels of collagen turnover markers are predictive of future events in the HFPEF population (albeit nonsignificantly so when adjusted for other variables) and that 6 months of angiotensin receptor blockade is insufficient to meaningfully reduce levels of these markers. These substudy findings with irbesartan were consistent with the neutral results of the I-PRESERVE (Irbesartan in Heart Failure With Preserved Systolic Function) study overall. The clinical implication of these findings is that if fibrosis and its sequelae are presumed to be important drivers of the disease process of HFPEF at the cellular level as well as contributory to future major cardiovascular events, then more specifically targeted blockade of fibrosis and its consequences is required. These targeted therapies may be through specific antifibrotic drugs as well as use of existing or novel agents that act at least in part through potent antifibrotic actions. Furthermore, in evaluating the clinical utility of therapies in HFPEF, assessment of the effect of these agents on fibrotic status of the heart may be mechanistically revealing.

# Relation of Peripheral Collagen Markers to Death and Hospitalization in Patients With Heart Failure and Preserved Ejection Fraction: Results of the I-PRESERVE Collagen Substudy

Henry Krum, Maros Elsik, Hans G. Schneider, Agata Ptaszynska, Marion Black, Peter E. Carson, Michel Komajda, Barry M. Massie, Robert S. McKelvie, John J. McMurray, Michael R. Zile and Inder S. Anand

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