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Extracellular cardiac matrix biomarkers in patients with reduced ejection fraction heart failure as predictors of response to cardiac resynchronisation therapy: a systematic review

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ABSTRACT

Objective Cardiac resynchronisation therapy (CRT) is an effective therapy for selected patients with heart failure (HF); however, a significant non-response rate exists. We examined current evidence on extracellular cardiac matrix (ECM) biomarkers in predicting response following CRT.

Methods Complete literature review of PubMed, Ovid SP MEDLINE, Cochrane Library and TRIP, reference lists, international cardiology conferences and ongoing studies between December 1999 and December 2015 conducted according to prospectively registered study selection and analysis criteria (PROSPERO:CRD42016025864) was performed. All observational and randomised control trials (RCT) were included if they tested prespecified ECM biomarkers' ability to predict CRT response. Risk of bias assessment and data extraction determined pooling of included studies was not feasible due to heterogeneity of the selected studies.

Results A total of 217 studies were screened; six (five prospective cohort and one RCT substudy) were included in analysis with 415 participants in total. Study sizes varied (n=55–260), cohort characteristics contrasted (male: 67.8%–83.6%, ischaemic aetiology: 40.2%–70.3%) and CRT response definitions differed (three clinical/functional, three echocardiographic). Consistent observation in all ECM biomarker behaviour before and after CRT implantation was not observed between studies. Lower type I and type III collagen synthesis biomarkers (N-terminal propeptides of type I and III procollagens) expression demonstrated replicated ability to predict reverse left ventricular remodelling.

Conclusion Collagen synthesis biomarkers offer the most potential as ECM biomarkers for predicting CRT response. Heterogeneity between these studies was large and limited the ability to pool and compare results numerically. Use of different response definitions was one of the biggest challenges.

KEY QUESTIONS

What is already known about this subject?

- Cardiac resynchronisation therapy (CRT) is associated with non-response in 20%–40% of selected patients with heart failure (HF). Selected vascular biomarkers are known to be associated with cardiac disease but it is unknown whether these can be used to predict CRT response.

What does this study adds?

- We performed a systematic review of all studies examining vascular biomarkers in CRT. We found that collagen synthesis biomarkers have the most potential for predicting CRT response, particularly N-terminal propeptides of type I and III procollagens. Matrix metalloproteinases-2 and 9 have no conclusive predictive value and need further investigation.

How might this impact clinical practice?

- Use of vascular biomarkers to predict CRT response could have enormous clinical benefit by selectively identifying those patients with HF who are likely to benefit. This has important implications for both patients and healthcare providers worldwide, especially given the current financial climate.

INTRODUCTION

Cardiac resynchronisation therapy (CRT) is an effective therapy for selected patients with heart failure (HF).[1 2] Current guidelines suggest that CRT is offered to those with a left ventricular ejection fraction (LVEF) ≤35% with resting 12-lead ECG QRS duration ≥150 ms or 120–149 ms with Left Bundle Branch Block (LBBB) morphology and refractory to optimal medical therapy (OMT).[3] CRT reduces mortality and improves morbidity, underpinned by reversal of pathophysiological adverse cardiac remodelling.[1 2] Unfortunately, a significant non-response rate of 20%–40% exists and has remained unchanged over the last decade, despite extensive research and investment.[1 2]

The extracellular cardiac matrix (ECM) is a dynamic support structure that remodels following cardiac injury and HF.[4 5] Progressive ECM remodelling is closely linked to HF severity and prognosis.[4 5] Cardiac collagen turnover alterations are central to the development and progression of cardiac fibrosis and HF.[5] Specific biomarkers of type I and type III collagen synthesis (N-terminal propeptides of type I and III procollagens (PINP and PIIINP),[6 7] carboxy-terminal propeptide of procollagen type I (PICP))[8 9] and degradation (carboxy-terminal telopeptide of type I collagen (ICTP or CITP))[9 10] products are associated with poor outcomes in HF. The proteolytic enzyme system matrix metalloproteinases (MMPs) and their regulators tissue inhibitors of MMPs (TIMPs) are involved in collagen degradation and have been implicated in HF development and progression.[4 5] Specifically, MMP-1,[11] a collagenase, MMP-2[12] and MMP-9,[13] both gelatinases and TIMP-1[11] are associated with HF outcomes. Galectin-3 (Gal-3) is a beta-galactoside-binding lectin released by activated cardiac macrophages, which are upregulated in HF, causing increased fibroblast proliferation, collagen deposition and ventricular dysfunction.[14] Gal-3 is strongly associated with inflammation and fibrosis with raised levels strongly predict poor HF outcomes.[14]

Turnover of ECM alters in HF and with reverse cardiac remodelling following CRT implantation may offer potential biomarkers for response prediction.[15] This systematic review examines the current evidence on the value of ECM biomarkers in predicting CRT response.

METHODOLOGY

Our systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.[16] It was prospectively registered with PROSPERO (CRD42016025864), an international registry of systematic reviews. A protocol was designed and implemented prospectively in-line with PRISMA-P 2015.[17]

Eligibility criteria

Strict eligibility criteria were applied to minimise heterogeneity of included articles. Observational studies (prospective or retrospective) and randomised control trials (RCTs) (including substudies) were included; basic science and review articles were excluded. Included study populations represented patients with HF meeting international CRT implant guidelines.[3] Studies had to be conducted on adults (age ≥18 years). Articles were included if they examined an ECM biomarker previously reported to predict HF outcomes.[4] Baseline ECM biomarkers, measured when patients were clinically stable prior to implantation, had to be compared with a predefined CRT 'response' criteria to evaluate their predictive value. Coronary sinus sampling and long-term trends in peripheral ECM biomarker behaviour were analysed if present.

A variety of clinical, functional or echocardiographic criteria and cardiovascular outcomes have been used to define CRT response in studies,[18] which often correlate poorly. All response criteria were included in the review. Cardiovascular outcomes could form part of a response definition or be presented separately; their absence was not an exclusion criterion.

Database search strategies

Detailed searches were conducted on PubMed, Ovid SP MEDLINE, Cochrane Library (CENTRAL) and TRIP in February 2016 by one author (CM) and reviewed by another independently (DA). The search strategy used specific terms (cardiac resynchronisation therapy/cardiac pacing/extracellular matrix) in combination, within titles/abstracts or Medical Subject Headings. Specific vascular biomarkers ('TIMP' 'MMP' 'collagen' 'Myostatin' 'Syndecan-4' and 'Galectin-3') were included in the search. A grey literature search involved searching the Clinical Trials database (www.clinicaltrials.gov) and international cardiology conferences (European Society of Cardiology, American Heart Association, American College of Cardiology) indexes for ongoing, abstracts and unpublished work. All included articles had their references searched for relevant publications. A date limitation of the last 15 years (31 December 1999–31 December 2015) was applied. No language restrictions were applied.

Title and abstract reviews were performed independently (CM/DA), consensus on eligibility criteria was required to be taken forward to full paper review; any conflicts were decided by an independent reviewer (FO). Duplications of articles or cohort use were identified and only the most relevant (decided by consensus) taken forward. The Critical Appraisal Skills Programme checklist (dependent on study design) was applied to full paper review to guide evaluation of article quality.[19] Consensus had to be reached on full paper reviews before being selected for inclusion; where consensus was not reached a third reviewer (FO) made the final decision. Contact was attempted with all included article authors and any others at full paper review that were indicated.

Data extraction and management

Full texts of included articles were obtained. Pilot data extraction was performed on two randomly selected articles and reviewed for robustness (CM, DA, FO, PB). A standardised data extraction form was created to collect data on each study's design (eligibility criteria, methodology, assessment period), patient population (numbers, age, gender, aetiology, ECG, left ventricular (LV) geometry, quality of life, New York Heart Association (NYHA), functional assessment), vascular biomarker/predictor (specific ECM surrogate biomarkers, units, conditions of sampling, laboratory assessment, statistical prediction model) and outcome (response definition and cardiovascular outcomes). Data extraction was performed by two independent

reviewers (CM/DA), a third independent reviewer (FO) resolved any disagreement.

Risk of bias assessment

Risk of bias for each study was assessed by two independent reviewers (CM, DA) utilising either the Risk of Bias Assessment Tool for Non-randomised Studies or the Cochrane Collaboration 'Risk of Bias' assessment tool.[20 21] Both have established criteria to examine selection bias, exposure measurement, blinding and completeness of outcome data.[20 21]

Data synthesis and analysis

A descriptive synthesis was performed to summarise findings of all selected articles. A meta-analysis of included study data for each specific ECM biomarker was not possible due to heterogeneity of outcome definitions and study designs. Evaluation of study designs, defined outcomes and cohort characteristics was performed. The same biomarkers compared in different included articles were compared. Continuous variables were summarised using the same units for each variable in the original text. Data were presented as mean ± standard deviation (SD), unless specified otherwise.

RESULTS

Figure 1 shows the screening and selection of published articles; 110 records were excluded after the screening stage as they did not meet inclusion criteria. Six articles met the inclusion criteria. Two abstracts[22 23] and one clinical trial entry (www.clinicaltrials.gov) (NCT15019908) were

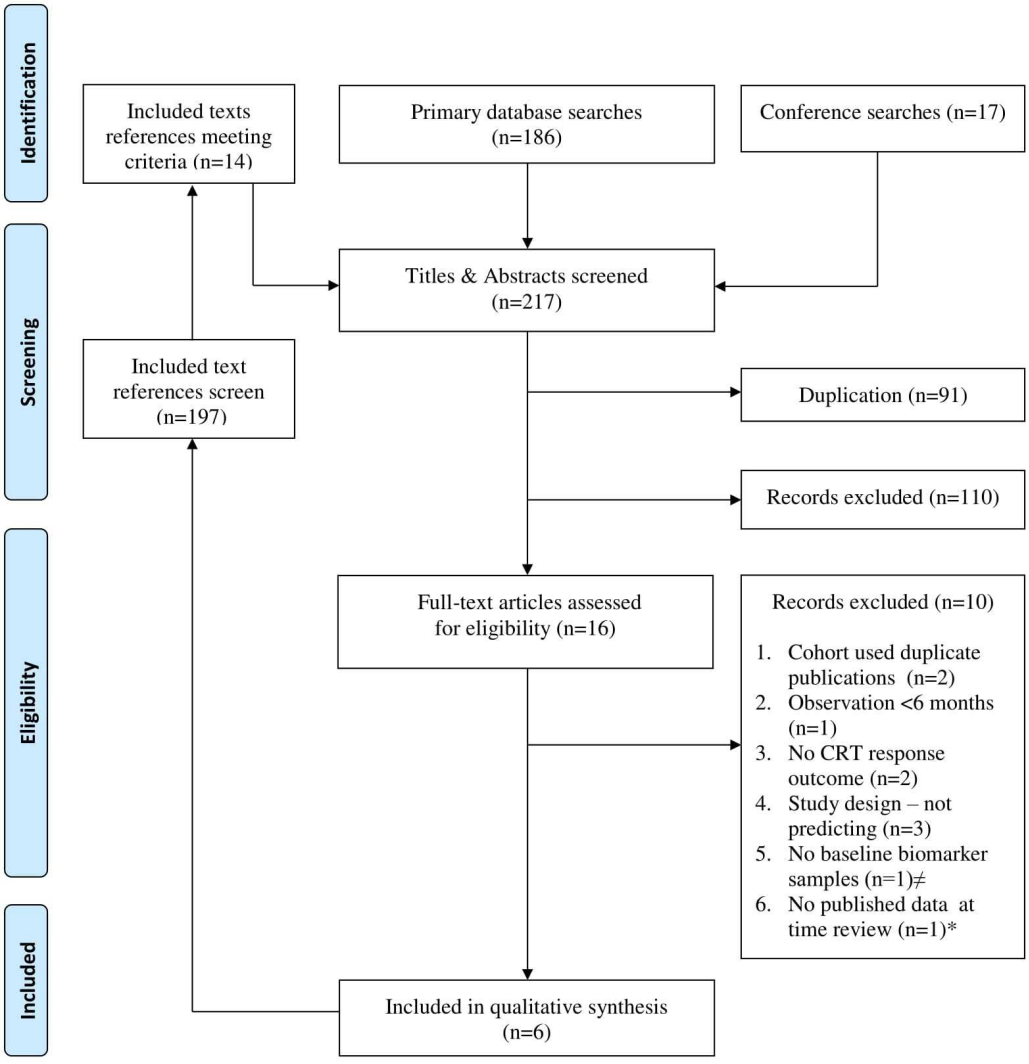

Figure 1 Flowchart of studies screening and selection. ≠Author contacted, poster presentation sent and no baseline extracellular cardiac matrix biomarker sample taken.[22] *Clinical trial (NCT15019908) author contacted and manuscript in preparation. CRT, cardiac resynchronisation therapy.

taken to full review (for potential inclusion). Related articles and information were sought, including contacting authors (all three kindly responded). None yet had articles published and additional information provided led to exclusion from review (no baseline biomarkers taken[22] or study design did not test biomarkers as predictors[23]).

Study design

Five prospective cohort studies and one RCT substudy[11] were included. Table 1 summarise the different study designs and CRT response outcome definitions used. Studies selected were published between 2008 and 2014. Risk of bias was assessed in each study using appropriate quality check tools. The lowest risk of bias was in the single RCT substudy.[11] The prospective cohort studies varied minimally in their bias assessment and none were excluded.

Garcia-Bolao *et al*[9] stated that 61 participants were consented; during the observation period there were four mortalities (three cardiac/one non-cardiac) and one functional assessment not performed at follow-up (6 min walk test not completed due to stroke). The cohort was 59 but no explicit statement about the two exclusions made. Lopez-Andres *et al*[11] published a substudy in 2012 of the 'The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure' (CARE-HF)[1] RCT which itself was published in 2005; interpretation of results is within this context. All studies included NYHA III–IV patients (mostly NYHA III). Two studies recruited NYHA II patients[24 25] with one also requiring a bradycardia pacing indication.[24] All studies included QRS duration >120 ms, except Garcia-Bolao *et al*[9] (QRS≥130 ms). In the CARE-HF trial, those with QRS duration 120–149 ms needed dyssynchrony on echocardiography.[1 8] All transvenous LV leads were implanted preferably to the most lateral position possible. Dong *et al*[26] performed only de novo CRT-defibrillator (CRT-d) implants. Three studies[10 24 26] commented on right ventricular lead placement with two[26] explicitly aiming for the right ventricular apex. In CARE-HF (and substudy), all had CRT-pacemaker (CRT-p) devices only.[1 11] CRT response definitions varied between included studies. Broadly, response definitions used were classified as three clinical and three echocardiographic. Reported response rates varied between 48.9% and 71.8% (table 1).

Baseline characteristics

The baseline characteristics of the patients are shown in table 2.

A total of 415 patients were included. The five prospective observational studies had mean age of 67±10 years[9 10] (Lopez-Andres *et al*[8] excluded as presented as median and IQR). There were 315 (75.9%) males in included studies, ranging 67.8%[9]–83.6%.[25] There was large variation in frequency of CRT-d/CRT-p implants in each study with two not providing this data.[10 25] One study included a high proportion of device upgrades[25]; the CARE-HF trial excluded upgrades,[1 8] the remaining four studies did not state upgrade status.[9 10 24 26] Atrial fibrillation (AF) was included in three prospective observational studies[8 24 25]; one did not report on AF or related publications.[10 27] Precise QRS duration was not stated in two studies.[24 26] Reporting of LV volumetric data varied between included studies. Three reported unadjusted LV end systolic volume (LVESV) and LV end diastolic volume (LVEDV) data which were similar to each other (table 3).[10 24 25] Dong *et al*[26] presented LVESV and LVEDV volume indexed figures only. Garcia-Bolao *et al*[9] provided LVEF only. LVEF was compared between the five prospective cohorts and showed similar mean LVEF between 25%–27%.[9 10 24–26]

Responder versus non-responders

Response status (responders vs non-responders (RvsNR)) was presented in four of the included studies.[9 10 24 26] Truong *et al*[25] did not provide characteristics of those defined by response. Lopez-Andres *et al*[8] outlined characteristics by allocation to CRT-p versus OMT, however, not by response. There were some baseline characteristic differences between the four studies for RvsNR[9 10 24 26]; Dong *et al*[26] demonstrated differences between RvsNR for LBBB status (15 (68.3%) vs 9 (39.1%), p=0.05) and ischaemic aetiology (9 (40.9%) vs 17 (73.9%], p=0.03). Tolosana *et al*[24] reported lower creatinine levels in RvsNR (1.25±0.3 mg/dL vs 1.76±0.8 mg/dL, p=0.01). Umar *et al*[10] reported that responders were older and had longer QRS duration than non-responders (age: 66±10 years vs 60±11 years, p=0.03; mean± standarderror QRS: 165±3 ms vs 135±8 ms, p=0.001). Notably, Hessel *et al* published a study using the same cohort as Umar *et al* and reported no difference in QRS duration for RvsNR (165±2 ms vs 153±3 ms, p=NS), suggesting one of these studies has recorded it incorrectly.[10 27]

ECM biomarkers

All ECM biomarker baseline concentrations and magnitude of association (if tested) are summarised in table 3. Lopez-Andres *et al*[8] did not provide baseline concentrations by response status, but comparison was made with the control group. Umar *et al*[10] showed baseline results for expression of ECM biomarkers studied. However, for PIIINP non-responders no baseline concentration was reported in the article, however no statistical significance is reported RvsNR.[10]

PINP/PICP

PINP and PICP share a 1:1 stoichiometric relationship with the collagen molecule; therefore, they were considered together. Umar *et al*[10] reported similar total cohort means values to Lopez-Andres *et al*[8] median values (the skew of this data is unknown). Umar *et al*[10] observed higher PINP baseline level predicted poor response. Garcia-Balao *et al*[9] reported the opposite for PICP. Lopez-Andres *et al*[8] observed no significant association of baseline PINP with CRT response or other outcomes. Variation in the pattern of reported levels between the

Table 1 Study designs and response outcome definitions

Study ID	Design	Participants recruited	HF/CRT participants	Inclusion criteria	Observation period	Assessments	ECM biomarkers	CRT responder definition	Response rate
Dong *et al*[26]	Prospective observational	65 (20 healthy controls)	45	LVEF≤35%, NYHA III–IV, QRS>120 ms, SR and OMT	6 months	Baseline/3 months/6 months: NYHA, 6MWT, TTE, blood samples	PIIINP	↓≥15% LVESVi and survived at 6 months	22 (48.9%)
Tolosana *et al*[24]	Prospective observational	55 (13 excluded after recruitment)	42	LVEF≤35%, NYHA III–IV, QRS>120 ms and OMT or cardiac pacing indication (LVEF<35%)	12 months	Baseline/6 months/12 months: NYHA, QoL (MLHFQ), 6MWT, TTE, ECG, blood samples	MMP-2, TIMP-1	↑>10% 6 MWT or if test not performed ↑>1 NYHA and survived/ no heart transplant at 12 months	25 (59.6%)
Truong *et al*[25]	Prospective observational	73	73	LVEF<35%, NYHA II–IV, QRS≥120 ms, OMT, HF decompensation <12 months	24 months (IQR 20.4–24.0)	Baseline: NYHA, ECG, TTE 1 month/3 months 6 months Clinical FU	Gal-3	Improvement HF clinical composite score[30] at 6 months	40 (54.7%)
Umar *et al*[10]	Prospective observational	64	64	LVEF<35%, NYHA III–IV, QRS>120 ms	6 months	Baseline/6 months: NYHA, TTE; QoL (MLHFQ), 6MWT, blood samples	PINP, PIIINP, ICTP, proMMP-1, TIMP-1	↓≥10% LVESV at 6 months	46 (71.8%)
Garcia-Bolao *et al* [9]	Prospective observational	61	59	LVEF≤35%, NYHA III–IV, LBBB, QRS≥130 ms, OMT	12 months	Baseline/12 months: NYHA, QoL (MLWHFQ), 6MWT, TTE, ECG, blood samples	PICP, CITP, MMP-1, MMP-2, MMP-9, TIMP-1	↑≥10% 6 MWT and survival from cardiac mortality at 12 months	35 (59.3%)
Lopez-Andres *et al*[8]*	Substudy randomised control trial: CARE-HF	260 (CARE-HF cohort available)	132 (CRT-P only)	LVEF<35%, NYHA III–IV, QRS>150 ms or 120–149 ms with echocardiographic dyssynchrony, OMT	Substudy: 18 months; CARE-HF: 29.4 months (range, 18.0–44.7)	Substudy Baseline/3 months/18 months: TTE, blood samples	PINP, PIIINP, ICTP, MMP-1, Gal-3	Survival and LVEF more than 35% at 18 months	CRT-p (n=108): 72 (66.6%); OMT (n=117): 103 (88.0%), p=0.0001

*Median (IQR).
CITP, carboxy-terminal telopeptide of collagen type-I; CRT, cardiac resynchronisation therapy; CRT-p, CRT-pacemaker; ECM, extracellular cardiac matrix; Gal-3, galectin-3; HF, heart failure; ICTP, carboxy-terminal telopeptide of type I collagen; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVESVi, left ventricular end-systolic volume indexed; 6MWT, six min walk test; MLHFQ, Minnesota living with heart failure questionnaire; MMP, metalloproteinases; NYHA, New York Heart Association; OMT, optimal medical therapy; PINP, N-terminal propeptides of type I procollagens; PIIINP, N-terminal propeptides of type III procollagens; QoL=quality of life; SR, sinus rhythm; TIMP, tissue inhibitors of MMPs; TTE, transthoracic echocardiogram.

Table 2 Baseline characteristics

Study ID	Age (years)	Male gender	CRT-d	Device upgrade	Ischaemic aetiology	Atrial fibrillation	Medication	LBBB	QRS (ms)	NYHA	6MWT (M)	LV
Dong *et al*[26]	68±9	37 (82.2%)	45 (100%)	Not reported	26 (57.8%)	Chronic AF excluded	ACEi/ARB 27 (60.0%), BB 41 (91.1%)	23 (53.3%)	>120	3.03±0.33	351±186	LVESVi 77±26 mL/m^2, LVEF 26%±5%
Tolosana *et al*[24]	66±8	35 (83.3%)	25 (59.5%)	Not reported	19 (45.2%)	8 (19%)	ACEi/ARB 33 (78.5%), BB 27 (64.3%), MRA 20 (47.6%)	Not reported	≥120	≥III=33 (78.5%) or II=9 (21.4%) → pacing indication	232±126	LVESV 162±63 mL, LVEDV 212±66 mL, LVEF 27%±7%
Truong *et al*[25]	68±12	61 (83.6%)	Yes	41 (56.2%)	39 (53.4%)	34 (46.5%)	ACEi/ARB 57 (78.1%), BB 64 (87.7%), MRA 16 (21.9%)	39 (53.4%)	168±27	2.9±0.4	Not done	LVESV 163±60 mL, LVEDV 226± 73 mL, LVEF 27%±7%
Umar *et al*[10]	64±11	52 (81%)	Yes	Not reported	45 (70.3%)	Not reported	Not reported	Not reported	162±24	3.1±0.2	330±114	LVESV 172±69 mL, LVEDV 229± 78 mL, LVEF 25%±8%
Garcia-Bolao *et al*[9]	69±4	40 (67.8%)	33 (55.9%)	Not reported	30 (50.8%)	11 (18.6%)	ACEi/ARB 59 (100%), BB 34 (57.6%), MRA 21 (35.6%)	51 (86.4%)	158±35	3.1±0.6	327±112	LVEF 25%±5%
Lopez-Andres *et al*[8]*	66 (59–71)	90 (68%)	0 (0%)	Excluded in CARE-HF	53 (40.2%)	AF excluded	ACEi/ARB 131 (99.2%), BB 88 (66.7%), MRA 73 (55.3%)	Not reported	160 (152–180)	3.0±0.2	Not done	(n=115) LVESV 206 mL (174–272), LVEDV 274 mL (233–355), LVEF 25% (21–29)

*Median (IQR).
ACEi, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BB, beta blocker; CRT-d, cardiac resynchronisation therapy defibrillator; CARE-HF, cardiac resynchronisation in heart failure; LBBB, left bundle branch block; LV, left ventricular; LVESV, left ventricular end-systolic volume; LVESVi, left ventricular end-systolic volume indexed; LVEDV, left ventricular end diastolic volume; 6MWT, six min walk test; MRA, mineralcorticoid receptor blocker; NYHA, New York Heart Association.

three studies were likely due to differences in response definitions and baseline characteristics. Garcia-Balao *et al*[9] utilised a clinical definition of response, whereas the other two studies used echocardiographic criteria.[8 10] All studies varied in duration of follow-up. Umar *et al*[10] had a higher proportion of men with ischaemic aetiology than the other studies. Lopez-Andres *et al*[8] excluded AF, whereas within the Garcia-Balao *et al*[9] cohort it was present in 18.6% of participants. Garcia-Balao *et al*[9] tested the predictive value of type I collagen turnover with the PICP:CITP ratio with a ratio ≥14.4 predicting response.

PIIINP

Variation was reported in trends of PIIINP levels at baseline. Dong *et al*[26] reported logarithmic figures making absolute figure comparison challenging. Geometric means could be calculated, but given small numbers of participants this was likely to underestimate the true mean.[26] Higher PIIINP levels in HF versus healthy controls (0.88±0.21 ug/L vs 0.71±0.14 ug/L, p=0.01) were observed.[26] Responders had significantly lower PIIINP baseline levels than non-responders (p=0.03).[26] Umar *et al*[10] demonstrated no difference in baseline levels between RvsNR. Lopez-Andres *et al*[8] reported similar baseline levels between CRT-p and OMT, but did observe PIIINP (>4.7 ug/L) in univariate analysis predicted cardiovascular outcomes (death or HF hospitalisation at 18 months) (OR 1.80, 95% CI 1.06 to 3.06, p=0.03).[8]

ICTP or CITP

Both ICTP and CITP were used to represent carboxyl-terminal peptides of type I collagen in three included studies. Umar *et al*[13] and Garcia-Balao *et al*[12] demonstrated similar baseline means for ICTP/CITP for the entire cohort. Neither identified independent predictors of CRT response.[9 10] Garcia-Bolao *et al*[9] identified that the PICP:CITP ratio strongly predicted response but was driven by PICP. Lopez-Andres *et al*[8] observed similar expression between CRT-p and OMT groups and showed no predictive value.

MMP-1, MMP-2 and MMP-9

There were variations in reported baseline concentrations for MMP-1. The mean for MMP-1 in Garcia-Bolao *et al*[9] was higher than median observed in CRT-p and OMT groups in Lopez-Andres *et al*,[8] though the data skew is unknown. Garcia-Bolao *et al*[9] examined the predictive value of MMP-1:TIMP-1, given their intrinsic regulatory role in collagen turnover,[5] but showed no statistical significance. Lopez-Andres *et al*[8] observed with a baseline MMP-1 ≤3 ug/L an adjusted threefold increased risk of CRT non-response and an increased risk of death or N-terminal *pro* B-type natriuretic peptide >1000 ng/L (OR 2.23, 95% CI 1.00 to 5.00, p=0.051/0.073 adjusted with/without renal function).[8] A precursor to MMP-1 called pro-MMP-1 (pro-MMP-1) was studied by Umar *et al*.[10] They observed no difference in baseline pro-MMP-1 expression between RvsNR.[10]

Table 3 Baseline ECM biomarkers concentrations between responders versus non-responders and their predictive value within included studies

ECM	Study ID	Baseline	Model	Predicting response
PINP	Umar *et al*[10]~	TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/L; NR: 41.9±4.3 ug/L, p=0.04	(Stepwise) multiple logistic regression‡	Univariate: OR 0.99, 95% CI 0.93 to1.00, p=0.05 Multivariable: OR 0.96, 95% CI 0.93 to 0.99, p=0.03
	Lopez-Andres *et al*[8]*	CRT-p: 33.0 ug/L (24.6–49.4); OMT: 33.1 ug/L (23.0–49.3), p=NS	(Stepwise) Multiple logistic regression†	No association with response
PICP	Garcia-Bolao *et al*[9]	TC: 74.3±29.9 ug/l; R: 85.6±29.4 ug/L; NR: 57.8±22.2 ug/L, p≤0.001	ROC:PICP:CITP	AUC 0.71, 95% CI 0.57 to 0.85; Cut-off 14.4,95% CI 9.8 to 17.7; Sensitivity 63% (51–80); Specificity 70% (50–85); OR 2.07, 95% CI 0.98 to 4.39
PIIINP	Dong *et al*[26]≠	TC: 0.88±0.21 ug/l; R: 0.80±0.20 ug/L; NR: 0.96±0.19 ug/L, p=0.03	(Stepwise) multiple logistic regression‡	Univariate: OR 0.77, 95% CI 0.62 to 0.97, p=0.03 Multivariable: OR 0.20, 95% CI 0.03 to 1.17, p=0.07
	Umar *et al*[10]~	R: 4.59±0.24 ug/L; NR: <responders, p=NS	(Stepwise) multiple logistic regression‡	Univariate: OR 1.23, 95% CI 0.86 to 1.76, p=0.23 Multivariable: OR 1.35, 95% CI 0.94 to 1.93, p=0.1
	Lopez-Andres *et al*[8]*	CRT-p: 4.6 ug/L (3.8–6.8); OMT: 4.7 ug/L (3.8–6.5), p=NS	(Stepwise) multiple logistic regression†	No association with response
ICTP	Umar *et al*[10]~	TC: 3.1±0.8 ug/l; R: 3.5±0.6 ug/L; NR: 2.1±0.4 ug/L, p=ns	(Stepwise) multiple logistic regression‡	Univariate: OR 1.24, 95% CI 0.93 to 1.66, p=0.13 Multivariable: No association with response
	Lopez-Andres *et al*[8]*	CRT-p: 4.1 ug/L (2.6–6.0); OMT: 3.4 ug/L (2.7–5.0), p=NS	(Stepwise) multiple logistic regression†	No association with response
CITP	Garcia-Bolao *et al*[9]	TC: 5.1±2.5 ug/l; R: 4.90±2.5 ug/L; NR: 5.3±2.5 ug/L, p=0.51	ROC:PICP:CITP	AUC 0.71,95% CI 0.57 to 0.85; Cut-off value 14.4, 95% CI 9.8 to 17.7; Sensitivity 63% (51–80); Specificity 70% (50–85); OR 2.07,95% CI 0.98to 4.39
ProMMP-1	Umar *et al*[10]~	TC: 7.7±0.8 ug/l; R: 7.6±0.7 ug/L; NR: 8.0±1.1 ug/L, p=0.71	(Stepwise) multiple logistic regression‡	Univariate: OR 0.97, 95% CI 0.87 to 1.09, p=0.71 Multivariable: No association demonstrated
MMP-1	Garcia-Bolao *et al*[9]	TC: 8.9±11.4 ug/l; R: 7.3±10.5 ug/L; NR: 11.3±12.5 ug/L, p=0.17	ROC	Not performed as no difference Baseline MMP-1:TIMP-1 ratio: 0.005±0.001, R: 0.004±0.0007 versus NR: 0.0063±0.0008, p=0.297
	Lopez-Andres *et al*[8]*	CRT-p: 2.7 ug/L (2.1–3.5); OMT: 2.7 ug/L (2.0–3.9), p=NS	(Stepwise) multiple logistic regression†	Univariate ≤3 ug/L: OR 2.42,95% CI 1.23 to 4.76, p=0.011 Multivariable ≤3 ug/L: OR 3.04,95% CI 1.37 to 6.71, p<0.01
MMP-2	Tolosana *et al*[24]	TC:295±70 ug/L; R:258±56 ug/L; NR:325±116 ug/L, p=0.02	Cox Regression Model‡	Univariate: difference already noted (p=0.02) Multivariable: No association demonstrated
	Garcia-Bolao *et al*[9]	TC: 1434±401.5 ug/l; R: 1393.8±374.5 ug/L; NR: 1496.6±438.9 ug/L, p=0.36	ROC	Not performed as no difference demonstrated
MMP-9	Garcia-Bolao *et al*[9]	TC: 44.7±23.2 ug/l; R: 41.1±22.8 ug/L; NR: 49.9±23.3 ug/L, p=0.17	ROC	Not performed as no difference demonstrated
TIMP-1	Tolosana *et al*[24]	TC:242±61 ug/l; R:216±50 ug/L; NR:277±59 ug/L, p=0.001	Cox Regression‡; ROC	Multivariate: OR 0.97, 95% CI 0.96 to 0.99, p=0.005, ROC: ≥248 ug/L, Sensitivity 71%, Specificity 72%, OR 6.8,95% CI 1.5 to 31
	Umar *et al*[10]~	TC: 120.3±8.2 ug/l; R: 124±5.2 ug/L; NR: 111±7.1 ug/L, p=0.16	(Stepwise) multiple logistic regression‡	Univariate: OR 1.01, 95% CI 0.99 to 1.03, p=0.16 Multivariable: No association demonstrated
	Garcia-Bolao *et al*[9]	TC: 488.9±249.5 ug/l; R: 437.5±136.5 ug/L; NR: 563.8±345.7 ug/L, p=0.135	ROC	Not performed as no difference. Baseline MMP-1:TIMP-1:TC: 0.005±0.001, R: 0.004±0.0007 versus NR: 0.0063±0.0008, p=0.297
Gal-3	Truong *et al*[25]*	TC: 18.1 ug/L (14.0–23.0), Positive result >25.9 ug/L	2×2 table; McNemar test	Peripheral: Sensitivity 15% (5–32), Specificity 80% (64–91), PPV: 38% (14–68), NPV: 53% (40–66)
	Lopez-Andres *et al*[8]*	CRTp: 25.7 ug/L (20.6–31.4); OMT: 25.1 ug/L (19.6–30.9), p=NS	(Stepwise) multiple logistic regression†	No association with response

*Median (IQR) given, data represented as CRT-p and OMT groups.
†Statistical model predicts 'non-response'.
‡Statistical model predicts 'response'.
~ mean±standard error.
≠biomarker mean±SD given in logarithmic form
AUC, area under the curve, CITP, carboxy-terminal telopeptide of collagen type-I; CRT-p, CRT-pacemaker; ECM, extracellular cardiac matrix; Gal-3, galectin-3; MMP, metalloproteinases; NPV, negative predictive value; NS=not significant; NR=non-responder; OMT, optimal medical therapy; PICP, carboxy-terminal propeptide of procollagen type I; PINP, N-terminal propeptides of type I procollagens; PIIINP, N-terminal propeptides of type III procollagens; PPV, positive predictive value; R, responder; ROC, receiver operator curve; TC, total cohort; TIMP, tissue inhibitors of MMPs.

Two studies reported cohort means for MMP-2 baseline concentration with large differences (table 3). Responders had lower MMP-2 baseline concentrations in both studies. Tolosana *et al*[24] reported a significant difference between RvsNR (p=0.02), whereas Garcia-Bolao *et al*[9] demonstrated no difference. The differences are not fully explained by study design, response definition or cohort characteristics as they showed similarities (tables 1 and 2). Variation in levels may be due to Tolosana *et al*[24] using plasma and Garcia-Bolao *et al*[9] using serum in their sandwich ELISAs. MMP-9 was reported by Garcia-Bolao *et al*,[9] who observed a trend towards lower baseline MMP-9 concentration for Responders. Baseline MMP-9 did not predict CRT response.[9]

TIMP-1

Tolosana *et al*[24] observed that responders had significantly lower concentrations at baseline of TIMP-1 than non-responders. Neither Umar *et al*[10] nor Garcia-Bolao *et al*[9] observed a significant difference in baseline TIMP-1 concentration between RvsNR. Higher peripheral TIMP-1 was identified as an independent predictor of non-response by Tolosana *et al*[24] in multivariable analysis; a concentration of ≥248 ug/L had a 71% sensitivity and 72% specificity for predicting non-response. However, Umar *et al*[10] did not identify TIMP-1 as a predictor. Garcia-Bolao *et al*[9] tested TIMP-1 in the MMP-1:TIMP-1 ratio and did not identify TIMP-1 as a significant predictor of RvsNR.

Gal-3

Lopez-Andres *et al*[8] reported higher baseline levels of Gal-3 than Truong *et al*,[25] due to different response definitions and variation in cohort characteristics. Lopez *et al*[8] used an echocardiographic definition at 18 months and Truong *et al*[25] utilised HF clinical composite score at 6 months. Truong *et al*[25] has higher ischaemic aetiology (53.4% vs 40.2%) and included patients with AF. Neither study reported baseline concentrations for RvsNR.[8 25] Truong *et al*[25] observed that peripheral baseline Gal-3 above a preset concentration (>25.9 ug/L) had low sensitivity and high specificity for predicting CRT response.

DISCUSSION

The ECM is a highly dynamic structure that is integral to myocardial structure and function which detrimentally remodels following cardiac injury leading to the altered turnover, replacing contractile tissue with collagen rich connective tissue and ultimately the development of myocardial fibrosis.[5] Myocardial fibrosis is characterised by adverse remodelling which contributes to systolic and diastolic HF.[5 28] PINP, PICP and PIIINP are released into the circulation during conversion and deposition of procollagen to collagen and are upregulated during myocardial fibrosis and associated with adverse HF outcomes.[5 7 15 28] Mechanistically, higher upregulation of collagen would challenge a CRT's ability to reverse remodel and for the patient to respond. Umar *et al*[10] supported this hypothesis observing significantly lower baseline PINP expression predicted echocardiographic response. Dong *et al*[26] did observe lower baseline PIIINP predicted echocardiographic response on univariate analysis, but not multivariable analysis. In contrast, Garcia-Balao *et al*[9] observed higher baseline expression of PICP in responders and PICP:CITP ratio (type I collagen turnover) of ≥14.4 had greater than twofold increased chance of predicting functional response, driven by PICP. Critically, echocardiographic and clinical/functional response criteria correlate poorly,[18] so could not be contrasted. Importantly, Lopez-Andres *et al*,[8] the largest study included in the review, did not observe upregulation of collagen synthesis predicting echocardiographic non-response, which does challenge the Umar *et al*[10] and Dong *et al*[26] observations; however, the cohort characteristics and study designs were different. The observations of collagen synthesis following CRT implantation conflict with each other. Umar *et al*[10] reported a significant increase in PINP and decrease in PIIINP expression in responders at 6 months; both would mechanistically be expected to be lower at follow-up. In contrast Garcia-Bolao *et al*[9] observed PICP levels decreased for responders and increased for non-responders at 1 year, which would be expected, but is based on a functional response definition. In contrast to collagen synthesis, degradation biomarkers (ICTP or CITP) did not predict CRT response.[8–10] Furthermore, no significant change in ICTP or CITP expression was observed at follow-up across all three studies.[8–10] Alteration in collagen synthesis rate is observed to be more powerful at predicting response than collagen degradation. Different patterns of collagen synthesis biomarkers predicting response have been observed; lower expression predicted LV reverse remodelling,[10 26] whereas higher rates predicted functional response.[9] The variation in these patterns is explained by the different response definitions and cohort characteristics. The study cohort for Umar *et al*[10] had a higher proportion of men and ischaemic cardiomyopathy than Garcia-Bolao *et al*.[9] The heterogeneities between these studies make drawing conclusions difficult. Lopez-Andres *et al*[8] also challenge any observations due to size of cohort and no prediction value to collagen turnover observed. Overall, collagen synthesis is observed to be important in predicting CRT response, especially LV reverse remodelling, with results replicated in two studies that lower rates predict LV reverse remodelling.[10 26]

MMP-1, MMP-2 and MMP-9 perform a critical role in myocardial collagen degradation and have been identified as being important prognostic markers in HF.[11 13 27] Predictive value for CRT non-response (death or LVEF ≤35% at 18 months) was only demonstrated in baseline MMP-1 expression ≤3 ug/l[8] supporting an observation by Jordan *et al*[11] that lower MMP-1 inferred worse HF prognosis. MMP-2 had large variations observed between the included studies,[8 24] but was not demonstrated to predict response. MMP-9 was only observed in one included study showing no predictive value[9];

however, recently Dini *et al*[13] demonstrated raised levels (>238 ng/mL) and predicted worse HF outcomes. MMP activity was not considered in any of these studies as a predictor but would be important to consider in the future. Current evidence suggests that MMPs, especially MMP-2 and MMP-9, have not yet had their potential fully evaluated.

TIMP-1 regulates the endogenous proteolytic MMP system involving discordant inhibition and in chronic inflammatory states stimulating collagen synthesis and myocardial fibrosis.[5 24] Tolosana *et al*[24] observed a significant baseline difference in RvsNR expression with lower TIMP-1 in responders. Tolosana *et al*[24] demonstrated that baseline TIMP-1 (≥248 ug/L) predicted CRT non-response. Trucco *et al*[29] in long-term follow-up of the same cohort demonstrated that the same threshold independently predicted mortality at 60±34 months (sensitivity 80% and specificity 71%). Tolosana *et al*[24] also demonstrated that statistically significant lower TIMP-1 is found in participants that do LV reverse remodel (LVESV reduction ≥10%). Umar *et al*[10] and Garcia-Bolao *et al*[9] observed no difference statistically at baseline. Variation between the reported literature in the magnitude of association of TIMP-1 exists; however, Tolosana *et al*[24] offers a well-designed prospective observational study which is powered giving strength to the conclusions drawn.

Gal-3 stimulates fibroblasts to release TIMPs and MMPs that regulate collagen turnover, resulting in myocardial fibrosis.[14] Elevated levels are independent predictors of adverse outcomes in HF.[14] Evaluation of Gal-3 as a predictor of response was limited, as RvsNR was not reported in either of the two studies.[8 25] Truong *et al*[25] demonstrated peripheral baseline Gal-3 ≥25.9 ug/L had specificity for predicting CRT response. Lopez-Andres *et al*[8] observed Gal-3 baseline expression ≥30 ng/L had nearly threefold increased risk of death or hospitalisation for worsening HF following CRT. Though not demonstrated to be a strong predictor, the evidence suggests that Gal-3 is a good biomarker for predicting poor outcomes in HF and needs further evaluation.

The greatest challenge for research into CRT response and one this review demonstrated is lack of an accepted response definition. Differing definitions rarely correlate,[18] which our review clearly demonstrates. Echocardiographic and clinical/functional definitions correlate very poorly and should never be compared or applied in a composite definition[18]; LV reverse remodelling should be considered separately.[18 30]

Study limitations

Heterogeneity among included studies was widespread despite a rigorous eligibility and screening criteria. The variations in study design, cohort characteristics and response definitions made pooling data in a meta-analysis impractical. CRT implantation techniques and indications have evolved over the last 15 years and offer another source of heterogeneity. Furthermore differences in laboratory techniques account for some variation among biomarker results. These limitations are particularly important to consider in future research studies.

CONCLUSIONS

Collagen synthesis biomarkers have shown the most potential, particularly PINP and PIIINP, but will require further study. MMP-2 and MMP-9 have no conclusive predictive value and need further investigation. Heterogeneity is the greatest challenge for research in this field and needs to be minimised in future studies. The most important initial step is for a universal response definition to be adopted and applied.

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REFERENCES

1. Cleland JG, Daubert JC, Erdmann E, *et al*. Cardiac resynchronization-heart failure study: the effect of cardiac resynchronization on morbidity and mortality in heart failure. *N Engl J Med* 2005;352:1539–49.
2. Bristow MR, Saxon LA, Boehmer J, *et al*. Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. *N Engl J Med* 2004;350:2140–50.
3. Tracy CM, Epstein AE, Darbar D, *et al*. 2012 ACCF/AHA/HRS focused update of the 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines. *Heart Rhythm* 2012;9:1737–53.
4. van Kimmenade RR, Januzzi JL. Emerging biomarkers in heart failure. *Clin Chem* 2012;58:127–38.
5. Spinale FG, Janicki JS, Zile MR. Membrane-associated matrix proteolysis and heart failure. *Circ Res* 2013;112:195–208.
6. Zannad F, Alla F, Dousset B, *et al*. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). Rales investigators. *Circulation* 2000;102:2700–6.
7. Cicoira M, Rossi A, Bonapace S, *et al*. Independent and additional prognostic value of aminoterminal propeptide of type III procollagen circulating levels in patients with chronic heart failure. *J Card Fail* 2004;10:403–11.
8. Lopez-Andrès N, Rossignol P, Iraqi W, *et al*. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: insights from the CARE-HF (Cardiac resynchronization in Heart failure) trial. *Eur J Heart Fail* 2012;14:74–81.

9. García-Bolao I, López B, Macías A, *et al*. Impact of collagen type I turnover on the long-term response to cardiac resynchronization therapy. *Eur Heart J* 2008;29:898–906.
10. Umar S, Bax JJ, Klok M, *et al*. Myocardial collagen metabolism in failing hearts before and during cardiac resynchronization therapy. *Eur J Heart Fail* 2008;10:878–83.
11. Jordán A, Roldán V, García M, *et al*. Matrix metalloproteinase-1 and its inhibitor, TIMP-1, in systolic heart failure: relation to functional data and prognosis. *J Intern Med* 2007;262:385–92.
12. George J, Patal S, Wexler D, *et al*. Circulating matrix metalloproteinase-2 but not matrix metalloproteinase-3, matrix metalloproteinase-9, or tissue inhibitor of metalloproteinase-1 predicts outcome in patients with congestive heart failure. *Am Heart J* 2005;150:484–7.
13. Dini FL, Buralli S, Bajraktari G, *et al*. Plasma matrix metalloproteinase-9 better predicts outcome than N-terminal protype-B natriuretic peptide in patients with systolic heart failure and a high prevalence of coronary artery disease. *Biomed Pharmacother* 2010;64:339–42.
14. Stolen CM, Adourian A, Meyer TE, *et al*. Plasma galectin-3 and heart failure outcomes in MADIT-CRT (multicenter automatic defibrillator implantation trial with cardiac resynchronization therapy). *J Card Fail* 2014;20:793–9.
15. Li M, Zhou Y, Zhou Y, *et al*. Improvement in collagen metabolism after 12 weeks' cardiac resynchronization therapy in patients with ischaemic cardiomyopathy. *J Int Med Res* 2013;41:200–7.
16. Moher D, Liberati A, Tetzlaff J, *et al*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;151:264–9.
17. Moher D, Shamseer L, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1.
18. Fornwalt BK, Sprague WW, BeDell P, *et al*. Agreement is poor among current criteria used to define response to cardiac resynchronization therapy. *Circulation* 2010;121:1985–91.
19. Critical Appraisal Skills Programme (CASP) Cohort Study Check list Oxford. 2013; (accessed 02 May 2014).http://www.casp-uk.net/#!casp-tools-checklists/c18f8
20. Higgins JP, Altman DG, Gotzsche PC, *et al*. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928.
21. Kim SY, Park JE, Lee YJ, *et al*. Testing a tool for assessing the risk of Bias for nonrandomized studies showed moderate reliability and promising validity. *J Clin Epidemiol* 2013;66:408–14.
22. Saba S, McTiernan C, Gutmann R, *et al*. London B: abstract 3252: clinical improvement Ater cardiac resynchronization therapy foretells lower serum levels of biomarkers reflecting cardiac Collagen deposition. *Circulation* 2009;120(Suppl 18):S771–S2.
23. Ducharme A, Prylutska H, Harel F, *et al*. 511 Similar remodeling benefit of LV-CRT and BiV-CRT in patients with heart failure and wide qrs: Insight from the evaluation of resynchronization therapy for heart failure (EARTH) trial. *Can J Cardiol* 2011;27:S248 http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/446/CN-01020446/frame.html
24. Tolosana JM, Mont L, Sitges M, *et al*. Plasma tissue inhibitor of matrix metalloproteinase-1 (TIMP-1): an independent predictor of poor response to cardiac resynchronization therapy. *Eur J Heart Fail* 2010;12:492–8.
25. Truong QA, Januzzi JL, Szymonifka J, *et al*. Coronary sinus biomarker sampling compared to peripheral venous blood for predicting outcomes in patients with severe heart failure undergoing cardiac resynchronization therapy: the BIOCRT study. *Heart Rhythm* 2014;11:2167–75.
26. Dong YX, Burnett JC, Chen HH, *et al*. Effect of cardiac resynchronization therapy on broad neurohormone biomarkers in heart failure. *J Interv Card Electrophysiol* 2011;30:241–9.
27. Hessel MH, Bleeker GB, Bax JJ, *et al*. Reverse ventricular remodelling after cardiac resynchronization therapy is associated with a reduction in serum tenascin-C and plasma matrix metalloproteinase-9 levels. *Eur J Heart Fail* 2007;9:1058–63.
28. Krum H, Elsik M, Schneider HG, *et al*. 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. *Circ Heart Fail* 2011;4:561–8.
29. Trucco E, Tolosana JM, Castel MA, *et al*. Guash E1, Matas M1, Arbelo E1, Berruezo A1, Brugada J1, Mont L1. plasma tissue inhibitor of matrix metalloproteinase-1 a predictor of long-term mortality in patients treated with cardiac resynchronization therapy. *Europace* 2016;18:232–7.
30. Packer M. Proposal for a new clinical end point to evaluate the efficacy of drugs and devices in the treatment of chronic heart failure. *J Card Fail* 2001;7:176–82.