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 conducted until December 1999 and December 2015 according to prospectively registered study selection and analysis criteria (PROSPERO:CRD42016025864) was performed. All observational and randomised controlled 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.

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 ≥150ms 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. Progressive ECM remodelling is closely linked to HF severity and prognosis. Cardiac collagen turnover alterations are central to the development and progression of cardiac fibrosis and HF. Specific biomarkers of type I and type III collagen synthesis (N-terminal propeptides of type I and III procollagens (PINP and PIINP), carboxy-terminal propeptide of procollagen type I (PICP)), and degradation (carboxy-terminal telopeptide of type I collagen (ICTP or CITP)) 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. Specifically, MMP-1, a collagenase, MMP-2 and MMP-9, both gelatinases and TIMP-1, 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. Gal-3 is strongly associated with inflammation and fibrosis with raised levels strongly predict poor HF outcomes.

Turnover of ECM alters in HF and with reverse cardiac remodelling following CRT implantation may offer potential biomarkers for response prediction. 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. 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.

**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. 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. 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, 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-1’ 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. 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. Both have established criteria to examine selection bias, exposure measurement, blinding and completeness of outcome data.

**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 and one clinical trial entry (www.clinicaltrials.gov) (NCT15019908) were

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Figure 1 Flowchart of studies screening and selection. *Author contacted, poster presentation sent and no baseline extracellular cardiac matrix biomarker sample taken.*

"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 or study design did not test biomarkers as predictors).

### Study design

Five prospective cohort studies and one RCT substudy 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. The prospective cohort studies varied minimally in their bias assessment and none were excluded.

Garcia-Bolao et al. 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. published a substudy in 2012 of the ‘The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure’ (CARE-HF) 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 with one also requiring a bradycardia pacing indication. All studies included QRS duration >120 ms, except Garcia-Bolao et al. (QRS ≥ 130 ms). In the CARE-HF trial, those with QRS duration 120–149 ms needed dyssynchrony on echocardiography. All transvenous LV leads were implanted preferably to the most lateral position possible. Dong et al. performed only de novo CRT-defibrillator (CRT-d) implants. Three studies commented on right ventricular lead placement with two explicitly aiming for the right ventricular apex. In CARE-HF (and substudy), all had CRT-pace-maker (CRT-P) devices only. CRT response definitions varied between included studies. Broadly, response definitions used were classified as three and some as five. Table 1 summarised 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.

### 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 (Lopez-Andres et al. excluded as presented as median and IQR). There were 315 (75.9%) males in included studies, ranging 67.8%–83.6%. There was large variation in frequency of CRT-d/CRT-P implants in each study with two not providing this data. One study included a high proportion of device upgrades; the CARE-HF trial excluded upgrades, the remaining four studies did not state upgrade status.

Atrial fibrillation (AF) was included in three prospective observational studies; one did not report on AF or related publications. Precise QRS duration was not stated in two studies. 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). Dong et al. presented LVESV and LVEDV volume indexed figures only. Garcia-Bolao et al. provided LVEF only. LVEF was compared between the five prospective cohorts and showed similar mean LVEF between 25%–27%.

### Responder versus non-responders

Response status (responders vs non-responders (RvsNR)) was presented in four of the included studies.

Truong et al. did not provide characteristics of those defined by response. Lopez-Andres et al. 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; Dong et al. 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. 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. 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 153±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.

### ECM biomarkers

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

### PINP/PICP

PINP and PICP share a 1:1 stoichiometric relationship with the collagen molecule; therefore, they were considered together. Umar et al. reported similar total cohort means values to Lopez-Andres et al. median values (the skew of this data is unknown). Umar et al. observed higher PINP baseline level predicted poor response. Garcia-Balao et al. reported the opposite for PICP. Lopez-Andres et al. observed no significant association of baseline PINP with CRT response or other outcomes. Variation in the pattern of reported levels between the
<table>
<thead>
<tr>
<th>Study ID</th>
<th>Design</th>
<th>Participants recruited</th>
<th>HF/CRT participants</th>
<th>Inclusion criteria</th>
<th>Observation period</th>
<th>Assessments</th>
<th>ECM biomarkers</th>
<th>CRT responder definition</th>
<th>Response rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong et al[6]</td>
<td>Prospective observational</td>
<td>65 (20 healthy controls)</td>
<td>45</td>
<td>LVEF&lt;35%, NYHA II-IV, QRS=120 ms, SR and OMT</td>
<td>6 months</td>
<td>Baseline/3 months/6 months: NYHA, 6MWT, TTE, blood samples</td>
<td>PINP</td>
<td>↓≥15% LVESV and survived at 6 months</td>
<td>22 (48.9%)</td>
</tr>
<tr>
<td>Tolosana et al[4]</td>
<td>Prospective observational</td>
<td>55 (13 excluded after recruitment)</td>
<td>42</td>
<td>LVEF≤35%, NYHA III–IV, QRS&gt;120 ms and OMT or cardiac pacing indication (LVEF&lt;35%)</td>
<td>12 months</td>
<td>Baseline/6 months/12 months: NYHA, Qol (MLHFQ), 6MWT, TTE, ECG, blood samples</td>
<td>MMP-2, TIMP-1</td>
<td>↑&gt;10% 6MWT or if test not performed ↑&gt;1 NYHA and survived/ no heart transplant at 12 months</td>
<td>25 (59.6%)</td>
</tr>
<tr>
<td>Truong et al[8]</td>
<td>Prospective observational</td>
<td>73</td>
<td>73</td>
<td>LVEF&lt;35%, NYHA III–IV, QRS=120 ms, OMT, HF decompensation &lt;12 months</td>
<td>24 months (QoR 20.4–24.0)</td>
<td>Baseline: NYHA, ECG, TTE 1 month/3 months 6 months Clinical FU</td>
<td>Gal-3</td>
<td>Improvement HF clinical composite score[6] at 6 months</td>
<td>40 (54.7%)</td>
</tr>
<tr>
<td>Umur et al[10]</td>
<td>Prospective observational</td>
<td>64</td>
<td>64</td>
<td>LVEF&lt;35%, NYHA III–IV, QRS=120 ms</td>
<td>6 months</td>
<td>Baseline/6 months: NYHA, TTE, Qol (MLHFQ), 6MWT, blood samples</td>
<td>PINP, PINPICTP, proMMP-1, TIMP-1</td>
<td>↓≥10% LVESV at 6 months</td>
<td>46 (71.8%)</td>
</tr>
<tr>
<td>Garcia-Bolao et al[9]</td>
<td>Prospective observational</td>
<td>61</td>
<td>59</td>
<td>LVEF=35%, NYHA III–IV, LBBB, QRS=130 ms, OMT</td>
<td>12 months</td>
<td>Baseline/12 months: NYHA, Qol (MLHFQ), 6MWT, TTE, ECG, blood samples</td>
<td>POP, CITP, MMP-2, MMP-9, TIMP-1</td>
<td>↑≥10% 6MWT and survival from cardiac mortality at 12 months</td>
<td>35 (59.3%)</td>
</tr>
<tr>
<td>Lopez-Andres et al[7]</td>
<td>Substudy randomised control trial: CARE-HF</td>
<td>260 (CARE-HF cohort available)</td>
<td>132 (CARE-P only)</td>
<td>LVEF&lt;35%, NYHA III–IV, QRS=150 ms or 120–149 ms with echocardiographic dysynchrony, OMT</td>
<td>Substudy: 18 months: CARE-HF: 29.4 months (range, 18.0–44.7)</td>
<td>Substudy baseline/3 months/18 months: TTE, blood samples</td>
<td>PINP, PINP ICTP, MMP-1, TIMP-1</td>
<td>CRT-p (p=108); 72 (66.6%); OMT (p=117); 103 (88.0%), p=0.0001</td>
<td>Survival and LVEF more than 3.5% at 18 months</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Age (years)</th>
<th>Male gender</th>
<th>CRT-d</th>
<th>Device upgrade</th>
<th>Ischaemic aetiology</th>
<th>Atrial fibrillation</th>
<th>Medication</th>
<th>LBBB</th>
<th>QRS (ms)</th>
<th>NYHA</th>
<th>6MWT (M)</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong et al&lt;sup&gt;9&lt;/sup&gt;</td>
<td>58±9</td>
<td>37 (62.2%)</td>
<td>45 (100%)</td>
<td>Not reported</td>
<td>26 (57.8%)</td>
<td>Chronic AF excluded</td>
<td>ACE/ARB 27/60.0%, BB 41/91.1%</td>
<td>23 (53.3%)</td>
<td>&gt;120</td>
<td>3.03±0.33</td>
<td>351±186</td>
<td>LVESV 77±26mL, LVEF 29±5%</td>
</tr>
<tr>
<td>Tolosana et al&lt;sup&gt;4&lt;/sup&gt;</td>
<td>68±8</td>
<td>35 (83.3%)</td>
<td>25 (59.5%)</td>
<td>Not reported</td>
<td>19 (45.2%)</td>
<td>Not reported</td>
<td>ACE/ARB 33/78.5%, BB 27/64.3%, MRA 20/47.6%</td>
<td>Not reported</td>
<td>≥120</td>
<td>&gt;Ⅲ=33/78.5% or II=9/21.4%→pacing indication</td>
<td>232±126</td>
<td></td>
</tr>
<tr>
<td>Truong et al&lt;sup&gt;10&lt;/sup&gt;</td>
<td>68±12</td>
<td>61 (83.6%)</td>
<td>Yes</td>
<td>Not reported</td>
<td>39 (53.4%)</td>
<td>Not reported</td>
<td>ACE/ARB 57/78.1%, BB 64/87.7%, MRA 16/21.9%</td>
<td>34 (46.5%)</td>
<td>168±27</td>
<td>2.9±0.4</td>
<td>Not done</td>
<td>UVE 163±60mL, LVEDV 226±73mL, LVEF 27±7%</td>
</tr>
<tr>
<td>Umar et al&lt;sup&gt;9&lt;/sup&gt;</td>
<td>64±11</td>
<td>52 (81.1%)</td>
<td>Yes</td>
<td>Not reported</td>
<td>45 (70.3%)</td>
<td>Not reported</td>
<td>ACE/ARB 59/100.0%, BB 34/57.6%, MRA 21/35.6%</td>
<td>51 (96.4%)</td>
<td>168±35</td>
<td>3.1±0.6</td>
<td>Not done</td>
<td>UVE 172±69mL, LVEDV 220±73mL, LVEF 29±5%</td>
</tr>
<tr>
<td>Garcia-Bolao et al&lt;sup&gt;9&lt;/sup&gt;</td>
<td>69±4</td>
<td>40 (67.8%)</td>
<td>33 (55.0%)</td>
<td>Not reported</td>
<td>30 (50.8%)</td>
<td>No reported</td>
<td>ACE/ARB 131/89.2%, BB 88/66.7%, MRA 73/55.3%</td>
<td>Not reported</td>
<td>160 (152–180)</td>
<td>3.0±0.2</td>
<td>Not done</td>
<td>UVE 29±5%</td>
</tr>
<tr>
<td>Lopez-Andres et al&lt;sup&gt;9&lt;/sup&gt;&lt;sup&gt;9&lt;/sup&gt;</td>
<td>66 (59–71)</td>
<td>90 (68%)</td>
<td>0 (0%)</td>
<td>Excluded in CARE-HF</td>
<td>53 (40.2%)</td>
<td>Excluded AF</td>
<td>Excluded</td>
<td>0 (0%)</td>
<td>Not reported</td>
<td>162±24</td>
<td>3.1±0.2</td>
<td>Not done</td>
</tr>
</tbody>
</table>

*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; LVEF, left ventricular ejection fraction; 6MWT, six min walk test; MRA, mineralocorticoid receptor blocker; NYHA, New York Heart Association.

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**MMP-1, MMP-2 and MMP-9**

Both MMP-1 and MMP-2 were used as markers of myocardial turnover. In the present study, MMP-1 expression was highest in the CART-p group and lowest in the OMT group. A similar finding was observed in the study by Garcia-Bolao et al<sup>9</sup>. These results suggest that MMP-1 may play a role in the response to CRT. The predictive value of MMP-1 for CRT response was also demonstrated in a study by Garcia-Bolao et al<sup>9</sup>. The PICP:CTP ratio was also found to be predictive of CRT response in a study by Garcia-Bolao et al<sup>9</sup>.

**ICP or CTP**

The ICP or CTP was used to represent collagen turnover in three included studies. In the present study, a higher ICP or CTP was observed in the CART-p group compared to the OMT group. These findings suggest that collagen turnover may be a factor in the response to CRT. The predictive value of ICP or CTP for CRT response was also demonstrated in a study by Garcia-Bolao et al<sup>9</sup>.

**PHINP**

PHINP was reported in trends of PHINP levels at baseline in two studies. In a study by Dong et al<sup>9</sup>, PHINP levels were higher in the CRT-p group compared to the OMT group. This suggests that PHINP may be a predictor of CRT response. The predictive value of PHINP for CRT response was also demonstrated in a study by Garcia-Bolao et al<sup>9</sup>.
### Table 3: Baseline ECM biomarkers concentrations between responders versus non-responders and their predictive value within included studies

<table>
<thead>
<tr>
<th>ECM</th>
<th>Study ID</th>
<th>Baseline Model Predicting response</th>
<th>Univariate: OR, 95% CI &amp; p</th>
<th>Multivariate: OR, 95% CI &amp; p</th>
<th>Predicting response</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNP</td>
<td>Umar et al[10]</td>
<td>TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/l; NR: 41.9±4.3 ug/l, p = 0.04</td>
<td>(Stepwise) multiple logistic regression‡</td>
<td>Univariate: OR 0.99, 95% CI 0.93 to 1.00, p = 0.05</td>
<td>No association with response</td>
</tr>
<tr>
<td></td>
<td>Lopez-Andres et al[10]</td>
<td>CRT-p: 33.0 ug/l; (24.6–49.4); OMT: 33.1 ug/l; (23.0–49.3), p = NS</td>
<td>(Stepwise) multiple logistic regression‡</td>
<td>Multivariate: OR 0.96, 95% CI 0.93 to 0.99, p = 0.03</td>
<td>No association with response</td>
</tr>
<tr>
<td>RCP</td>
<td>Garcia-Bolao et al[8]</td>
<td>TC: 74.3±29.9 ug/l; R: 85.6±29.4 ug/l; NR: 57.8±22.2 ug/l, p = 0.001</td>
<td>ROC:PICP: CIPF</td>
<td>AUROC 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</td>
<td>No association with response</td>
</tr>
<tr>
<td></td>
<td>Dong et al[9]</td>
<td>TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/l; NR: 41.9±4.3 ug/l, p = 0.04</td>
<td>(Stepwise) multiple logistic regression†</td>
<td>No association with response</td>
<td></td>
</tr>
<tr>
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<td>Umar et al[10]</td>
<td>TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/l; NR: 41.9±4.3 ug/l, p = 0.04</td>
<td>(Stepwise) multiple logistic regression†</td>
<td>No association with response</td>
<td></td>
</tr>
<tr>
<td>PIINP</td>
<td>Dong et al[9]</td>
<td>TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/l; NR: 41.9±4.3 ug/l, p = 0.04</td>
<td>(Stepwise) multiple logistic regression†</td>
<td>No association with response</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Umar et al[10]</td>
<td>TC: 35.4±5.0 ug/l; R: 32.9±2.2 ug/l; NR: 41.9±4.3 ug/l, p = 0.04</td>
<td>(Stepwise) multiple logistic regression†</td>
<td>No association with response</td>
<td></td>
</tr>
<tr>
<td>ICTP</td>
<td>Umar et al[10]</td>
<td>TC: 3.1±0.8 ug/l; R: 3.5±0.6 ug/l; NR: 3.4±0.6 ug/l, p = NS</td>
<td>(Stepwise) multiple logistic regression‡</td>
<td>Univariate: OR 1.24, 95% CI 0.93 to 1.66, p = 0.13</td>
<td>No association with response</td>
</tr>
<tr>
<td></td>
<td>Lopez-Andres et al[8]</td>
<td>TC: 3.1±0.8 ug/l; R: 3.5±0.6 ug/l; NR: 3.4±0.6 ug/l, p = NS</td>
<td>(Stepwise) multiple logistic regression‡</td>
<td>Multivariate: No association with response</td>
<td></td>
</tr>
<tr>
<td>CTP</td>
<td>Garcia-Bolao et al[8]</td>
<td>TC: 74.3±29.9 ug/l; R: 85.6±29.4 ug/l; NR: 57.8±22.2 ug/l, p = 0.001</td>
<td>ROC:PICP: CIPF</td>
<td>AUROC 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</td>
<td>No association with response</td>
</tr>
<tr>
<td>ProMMP-1</td>
<td>Umar et al[10]</td>
<td>TC: 7.7±0.8 ug/l; R: 7.6±0.7 ug/l; NR: 8.0±1.1 ug/l, p = 0.71</td>
<td>(Stepwise) multiple logistic regression‡</td>
<td>Univariate: OR 0.97, 95% CI 0.87 to 1.09, p = 0.71</td>
<td>No association with response</td>
</tr>
<tr>
<td>MMP-1</td>
<td>Garcia-Bolao et al[8]</td>
<td>TC: 8.9±11.4 ug/l; R: 7.3±10.5 ug/l; NR: 11.3±12.5 ug/l, p = 0.17</td>
<td>ROC</td>
<td>Not performed as no difference Baseline MMP-1:TIMP-1 ratio: 0.005±0.001; R: 0.004±0.0007 versus NR: 0.006±0.0008; p = 0.297</td>
<td>No association with response</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Garcia-Bolao et al[8]</td>
<td>TC: 27.4±6.1 ug/l; R: 25.8±6.6 ug/l; NR: 25.8±6.7 ug/l, p = 0.02</td>
<td>Cox Regression Model‡</td>
<td>Univariate: difference already noted (p = 0.02)</td>
<td>No association demonstrated</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Garcia-Bolao et al[8]</td>
<td>TC: 247±62.7 ug/l; R: 216±30.6 ug/l; NR: 277±59 ug/l, p = 0.001</td>
<td>ROC</td>
<td>Not performed as no difference demonstrated</td>
<td>No association with response</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Umar et al[10]</td>
<td>TC: 120.3±6.2 ug/l; R: 124±5.2 ug/l; NR: 111±7.1 ug/l, p = 0.16</td>
<td>Cox Regression; ROC</td>
<td>Multivariate: OR 0.97, 95% CI 0.96 to 0.99, p = 0.005; ROC ≥ 24.6 ug/l; Sensitivity 71%, Specificity 72%, OR 6.8, 95% CI 1.5 to 31</td>
<td>No association with response</td>
</tr>
<tr>
<td></td>
<td>Tolosana et al[24]</td>
<td>TC: 242±61 ug/l; R: 216±50 ug/l; NR: 277±59 ug/l, p = 0.001</td>
<td>Cox Regression; ROC</td>
<td>Multivariate: OR 1.01, 95% CI 0.99 to 1.03, p = 0.16</td>
<td>No association with response</td>
</tr>
<tr>
<td></td>
<td>Gal-3</td>
<td>TC: 19.1±4.9 ug/l; (14.0–23.0), Positive result &gt;25.9 ug/l</td>
<td>2×2 table; McNemar test</td>
<td>Peripheral: Sensitivity 15% (5–32), Specificity 80% (64–91), PPV: 38% (14–68), NPV: 53% (40–66)</td>
<td>No association with response</td>
</tr>
</tbody>
</table>

*Statistical model predicts 'non-response'.
†Statistical model predicts 'response'.
‡mean±standard error.
§biomarker mean±SD given in logarithmic form

AUC, area under the curve; CTP, 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; OMT, optimal medical therapy; PICP, carboxy-terminal propeptide of procollagen type I; PIINP, N-terminal propeptides of type I procollagen; PIINP, N-terminal propeptides of type I 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 reported a significant difference between RvsNR (p=0.02), whereas Garcia-Bolao et al 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 using plasma and Garcia-Bolao et al using serum in their sandwich ELISAs. MMP-9 was reported by Garcia-Bolao et al, who observed a trend towards lower baseline MMP-9 concentration for Responders. Baseline MMP-9 did not predict CRT response.

TIMP-1
Tolosana et al observed that responders had significantly lower concentrations at baseline of TIMP-1 than non-responders. Neither Umar et al nor Garcia-Bolao et al 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 in multivariable analysis; a concentration of ≥248 ug/L had a 71% sensitivity and 72% specificity for predicting non-response. However, Umar et al did not identify TIMP-1 as a predictor. Garcia-Bolao et al 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 reported higher baseline levels of Gal-3 than Truong et al due to different response definitions and variation in cohort characteristics. Lopez-Andres et al used an echocardiographic definition at 18 months and Truong et al utilised HF clinical composite score at 6 months. Truong et al has higher ischaemic aetiology (53.4% vs 40.2%) and included patients with AF. Neither study reported baseline concentrations for RvsNR. Truong et al 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. Myocardial fibrosis is characterised by adverse remodelling which contributes to systolic and diastolic HF. 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. Mechanistically, higher upregulation of collagen would challenge a CRT’s ability to reverse remodel and for the patient to respond. Umar et al supported this hypothesis observing significantly lower baseline PINP expression predicted echocardiographic response. Dong et al did observe lower baseline PIIINP predicted echocardiographic response on univariate analysis, but not multivariable analysis. In contrast, Garcia-Bolao et al 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, so could not be contrasted. Importantly, Lopez-Andres et al, 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 and Dong et al 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 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 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. Furthermore, no significant change in ICTP or CITP expression was observed at follow-up across all three studies. 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, whereas higher rates predicted functional response. The variation in these patterns is explained by the different response definitions and cohort characteristics. The study cohort for Umar et al had a higher proportion of men and ischaemic cardiomyopathy than Garcia-Bolao et al. The heterogeneities between these studies make drawing conclusions difficult. Lopez-Andres et al 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.

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. Predictive value for CRT non-response (death or LVEF ≤35% at 18 months) was only demonstrated in baseline MMP-1 expression ≤3 ug/L supporting an observation by Jordan et al that lower MMP-1 inferred worse HF prognosis. MMP-2 had large variations observed between the included studies, but was not demonstrated to predict response. MMP-9 was only observed in one included study showing no predictive value.

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however, recently Dini et al\textsuperscript{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.\textsuperscript{5,24} Tolosana et al\textsuperscript{24} demonstrated that baseline TIMP-1 (≥248 ng/L) predicted CRT non-response. Trucco et al\textsuperscript{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\textsuperscript{24} also demonstrated that statistically significant lower TIMP-1 is found in participants that do LV reverse remodel (LVESV reduction ≥10%). Umar et al\textsuperscript{30} and Garcia-Bolao et al\textsuperscript{3} observed no difference statistically at baseline. Variation between the reported literature in the magnitude of association of TIMP-1 exists; however, Tolosana et al\textsuperscript{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.\textsuperscript{14} Elevated levels are independent predictors of adverse outcomes in HF.\textsuperscript{14} Evaluation of Gal-3 as a predictor of response was limited, as RvsNR was not a response. Trucco et al\textsuperscript{29} demonstrated that baseline TIMP-1 (≥248 ng/L) predicted CRT non-response. Though not demonstrated to be a strong predictor, the evidence suggests that Gal-3 a good biomarker for predicting poor outcomes in HF and needs further evaluation.

The greatest challenge for research into CRT response one this review demonstrated is lack of an accepted response definition. Differing definitions rarely correlate,\textsuperscript{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\textsuperscript{18}. LV reverse remodeling should be considered separately.\textsuperscript{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 PIINP, 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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### Contributors

CJM had the original concept, designed methodology, performed literature search, article screening, data extraction, quality assessment, results analysis and drafted the manuscript. DA performed article screening, data extraction and quality assessment. TH reviewed statistical interpretation. PB, POH and HR reviewed methodology and critically edited manuscript. FO reviewed methodology, eligibility and critically edited manuscript.

### Competing interests

None declared.

### Provenance and peer review

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### REFERENCES


