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ORIGINAL ARTICLE

Significance of Anti-Myosin Antibody Formation in Patients With Myocardial Infarction: A Prospective Observational Study

Tom J. O'Donohoe, MBBS [a,b,d,e*], Ryan G. Schrale, MBBS, FCSANZ [b,c], Suchandan Sikder, DVM, MS [a,d], Nuzhat Surve, BSc, MSc [a,f], Donna Rudd, PhD [a,d], Natkunam Ketheesan, MD, PhD [a,b,d,g]

[a]Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Qld, Australia
[b]College of Medicine and Dentistry, James Cook University, Townsville, Qld, Australia
[c]Cardiac Services, Townsville Hospital, Townsville, Qld, Australia
[d]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Qld, Australia
[e]St. Vincent's Hospital, Melbourne, Vic, Australia
[f]Seth GS Medical College and KEM Hospital, Mumbai, India
[g]University of New England, Newcastle, NSW, Australia

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Background Anti-myosin antibodies (AMAs) are often formed in response to myocardial infarction (MI) and have been implicated in maladaptive cardiac remodelling. We aimed to: (1) compare AMA formation in patients with Non-ST-Elevation MI (NSTEMI) and ST-Elevation MI (STEMI); (2) evaluate factors predicting autoantibody formation; and, (3) explore their functional significance.

Methods Immunoglobulin M (IgM) and Immunoglobulin G (IgG) AMA titres were determined in serum samples collected at admission, 3 and 6 months post MI. The relationship between demographic and clinical data, and antibody formation, was investigated to determine factors predicting antibody formation and functional significance.

Results Forty-three (43) patients were consecutively recruited; 74.4% were positive for IgM at admission, compared with 23.3% for IgG. Mean IgG levels increased by 1.24% (±0.28) at 3 months, and 13.55% (±0.13) at 6 months post MI. Mean antibody levels were significantly higher in the NSTEMI cohort at both follow-up time points for IgG ($p < 0.001$, $p < 0.0001$), but not IgM ($p = 0.910$, $p = 0.066$). A moderately positive correlation between infarct size and increase in mean IgM concentration was observed at 3 months ($r(98) = 0.455$; $p = 0.015$). Anti-myosin antibody formation was not associated with an unfavourable outcome at follow-up.

Conclusions Anti-myosin antibodies are formed in a significant proportion of patients following MI, particularly among those with NSTEMI. While IgM levels fall after infarction, IgG levels increase and persist beyond 6 months of follow-up. This raises the possibility that they may contribute to long-term myocardial damage and dysfunction. Future research should focus on the specific epitopes that are targeted by these antibodies, and their functional significance. This may result in the emergence of novel therapies to attenuate cardiac dysfunction in MI patients.

Keywords Myocardial infarction • Ischaemic heart disease • Anti-myosin antibodies • Autoantibodies • Autoimmunity

*Corresponding author at: St. Vincent's Hospital, 41 Victoria Parade, Fitzroy, Vic 3065, Australia. Tel.: +61 (0) 3 9231 2211., Email: thomas.odonohoe@svha.org.au
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Introduction

Myocardial infarction (MI) is followed by a process of pathological ventricular remodelling, which may compound contractile dysfunction and contribute to congestive cardiac failure. Despite significant scientific scrutiny, the pathophysiological basis of this process is incompletely understood, and we remain largely unable to arrest or reverse it. One hypothesis suggests that functionally significant antibodies, formed in response to leaked cardiac proteins—including cardiac myosin—may make an important contribution [1,2].

Anti-myosin antibodies (AMA), usually directed against the S2 portion of the myosin protein [3–6], have been described in healthy individuals [7–14] and patients with dilated cardiomyopathy (DCM) [13–17], rheumatic heart disease [4,18], myocarditis [8,15,19] and myocardial infarction [10,11,20,21,22]. When isolated from human patients with MI and DCM, these antibodies have been demonstrated to significantly reduce myocyte contractility [23], increase cytoplasmic calcium concentrations and induce cardiomyocyte apoptosis in experimental animals [24]. Monoclonal AMAs have also been shown to deposit in the myocardium and induce myocarditis in some strains of mice [25,26].

Given that cardiac myosin is an exclusively intracellular protein, it has been suggested that AMA mediated damage must be confined to areas of cardiac damage. However, immunofluorescence studies do not support this hypothesis [23] and, in animal models, it appears that AMAs exert their effect by stimulating β-adrenergic receptors (β-AR) on the cardiomyocyte surface [27]. Li et al. have causally associated this AMA mediated β-AR stimulation with left ventriculomegaly, and atrophy of the interventricular septum and posterior ventricular wall of experimental rats [27,28].

Despite this compelling animal data, the formation and functional significance of AMA in patients with MI has not previously attracted significant scientific attention and remains poorly understood. No factors have been reliably demonstrated to predict AMA formation and the functional significance of these antibodies is unclear. Therefore, we sought to evaluate the demographic and clinical factors predicting AMA formation and to explore the relationship between these antibodies and patient outcomes in the first 6 months following MI.

Materials and Methods

This study had a prospective, observational design and was conducted at a tertiary cardiac centre in Queensland, Australia between 24 June 2014 and 24 February 2015. Consent was sought from all patients presenting to the Emergency Department with a diagnosis of Non-ST-Elevation MI (NSTEMI) or ST-Elevation MI (STEMI), based upon the European Society of Cardiology (ESC)/American College of Cardiology Foundation (ACCF)/American Heart Association (AHA)/World Heart Federation (WHF) universal definition of MI [29]. Participation was limited to adults (age > 18) who had experienced a spontaneous (Type-1) MI. Eligibility was also restricted to patients that presented to hospital within 24 hours of symptom onset, and those in whom serum samples could be obtained within 48 hours of admission. Patients with impaired ability to consent, metastatic malignancy and dilated cardiomyopathy (DCM) were all excluded. This study was approved by the Human Research Ethics Committee for the participating hospital (HREC/14/ATHS/15, SSA/14/QTHS/107).

Demographic data were obtained to identify baseline cardiovascular risk, functional capacity and previous cardiac insults, which may have resulted in intracellular protein leakage and, potentially, AMA formation (Table 1). Clinical, laboratory, sonographic and angiographic information were obtained from patient notes and the relevant electronic databases. Continuous data were recorded as exact values, while dichotomous outcomes were coded after extraction.

Serum was isolated from blood collected via peripheral venipuncture. These samples were used to determine cardiac troponin I (cTnI), creatine kinase MB (CK-MB), B-Type Naturetic Peptide (BNP) and AMA (IgM and IgG) levels. Patients were followed-up at 3 and 6 months following infarction and serum samples and clinical data were obtained at these time points.

Laboratory Methodology

Anti-Myosin Antibodies

Microtitre plates (96-well Maxisorp plates Nunc, Thermo Scientific, Waltham, MA, USA) were coated with porcine cardiac myosin (Sigma Aldrich, St Louis, MO, USA) at a concentration of 10 μg/mL, and refrigerated for 24 hours at 4 °C. After removal of the coating antigen, wells were filled with 200 μl of a blocking buffer (1% Bovine Serum Albumin (BSA) in PBS, Sigma Aldrich, St Louis, MO, USA), and plates were incubated for 24 hours at 37 °C. Serum samples were then diluted to a concentration of 1:10, and added to microtitre plates in duplicate. Participant samples were accompanied by a positive control, a negative control, and two wells with wash buffer alone. Plates were then incubated at 37 °C for 1 hour. Following incubation, plates were rinsed in wash buffer, and 100 μL aliquots of HRP-goat anti-human IgG (Jackson Immunoresearch Laboratories, Cat# 109-035-088, West Grove, PA, US) and HRP-goat anti-human IgM (Jackson Immunoresearch Laboratories Cat#109-035-125, West Grove, PA, USA), diluted to 1:20000, were added to each well. The washing step was repeated following an additional 1-hour incubation at 37 °C. 100 μL aliquots of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) substrate were then added to each well, and plates were incubated at room temperature for 30 minutes. Optical density (OD) was measured using an automated microtitre plate reader (EZ Read 2000, Biochrom, Cambridge, UK) at dual wavelengths: 405 nm and 492 nm.

Sample positivity was defined as any result greater than two standard deviations above the mean OD of the negative control.

Table 1 Baseline Characteristics.

Demographics	**[n (%)]**
Mean Age	59.9 (± 11.2)
Men	32 (74.4)
Mean BMI	30.0 (±6.1)
Cardiovascular Risk Factors	[n (%)]
Hypertension	28 (65.1)
Diabetes mellitus	12 (27.9)
Dyslipidaemia	25 (58.1)
Smoking	
Current	15 (34.9)
Mean pack years	20.07 (±21.9)
Family History of MI	19 (44.2)
Cardiovascular History	[n (%)]
Angina	12 (27.9)
MI	11 (25.6)
PCI	8 (18.6)
AF	2 (4.7)
Clinical Characteristics	[n (%)]
Mean Time to Pres	4.30 (± 4.5)
MI Type	
STEMI	21 (48.8)
NSTEMI	22 (51.2)
Admission NYHA	
NYHA I	36 (83.7)
NYHA II	3 (7.0)
NYHA III	4 (9.3)
NYHA IV	0 (0.0)
Presentation PO	5 (11.6)
VT/Cardiac arrest	1 (2.3)
Thrombolysis*	8 (38.1)
Dual antiplatelets	35 (81.4)
Biochemical Characteristics	**[$\bar{x}$, {s)]**
Troponin	
Admission	0.8 (±2.6)
Peak	34.5 (±72.6)
AUC	58.3 (±130.5)
Creatine kinase	
Admission	1031.8 (±1738.1)
Peak	1210.2 (±2070.1)
BNP	62.50 (±55.8)
Hb	145.0 (±14.8)
eGFR	78.1 (±13.4)
TC	4.9 (±1.2)
Imaging	
Echocardiography	[$\bar{x}$, {s)]
EF [$\bar{x}$, {s)]	53.7 (±11.6)
LVIDd [$\bar{x}$, {s)]	4.9 (±0.6)
LVIDs [$\bar{x}$, {s)]	3.4 (±0.7)
IVS [$\bar{x}$, {s)]	1.0 (±0.2)
Post. Wall [$\bar{x}$, {s)]	1.0 (±0.2)

Table 1. (continued).

Biochemical Characteristics	**[$\bar{x}$, {s)]**
EDV [$\bar{x}$, {s)]	95.4 (±23.3)
E:E′ [$\bar{x}$, {s)]	10.3 (±3.2)
Angiography	[n (%)]
Duke Jeopardy Score	
$\bar{x}$, {s)	4 (2-6)
0	3 (7.0)
2	13 (30.2)
4	10 (23.3)
6	10 (23.3)
8	3 (7.0)
10	1 (2.3)
12	0 (0.0)
Infarct Location	
Anterior	15 (44.1)
Inferior	12 (35.3)
Lateral	7 (16.3)

*Applies only to STEMI patients.

Abbreviations: BMI, body mass index; MI, myocardial infarction; PCI, percutaneous coronary intervention; AF, atrial fibrillation; RHD, rheumatic heart disease; NYHA, New York Heart Association; VT, ventricular tachycardia; AUC, area under the curve; BNP, B-type naturetic peptide; Hb, haemaglobin; eGFR, estimated Glomerular Filtration Rate; TC, total cholesterol; EF, ejection fraction; LVIDd, left-ventricular internal diameter (diastole); LVIDs, left-ventricular internal diameter (systole); IVS, interventricular septum; EDV, end-diastolic volume; E:E′, E:E′ ratio.

Cardiac Troponin I, Creatine-kinase MB and B-type Naturetic Peptide

Cardiac troponin I levels were determined using a Beckman Coulter Access 2 Immunoassay System (Brea, CA, USA), CK-MB measurements were carried out on a Beckman Coulter AU480 Chemistry System, and BNP concentration was determined using a point of care, automated ELISA (Abbott Laboratories, Green Oaks, IL, USA). All systems underwent routine maintenance and calibration prior to analysis.

Statistical Methodology

All analyses were carried out with Statistical Package for the Social Sciences (SPSS) Version 23 (IBM Corporation, Armonk, NY, USA). Graphs were produced using GraphPad Prism 6.0 (GraphPad Software Inc, La Jolla, CA, USA) and Microsoft Excel 2012 (Microsoft Corporation, Redmond, WA, USA). A p-value of <0.05 was considered to be statistically significant. Dichotomous variables were analysed using a Fisher's Exact test, or Pearson's Chi-Square, according to independence. Where only two groups were under comparison, normally distributed continuous data were analysed using the independent-samples T-test or Welch's T-test, according to variance. A Mann-Whitney U Test was used to compare median values of non-parametric

data. For multiple comparisons, a one-way analysis of variance (ANOVA) was undertaken. Where appropriate, the presence and direction of a linear relationship between continuous variables was determined using the Pearson product-moment correlation. The strength of the association was described as strong, moderate or small according to the coefficient (r) value. As described by Cohen, coefficient values of 0.1–0.3 are considered small in strength, with 0.3–0.5 described as moderate and >0.5 as strong [30].

Results

Participants

A total of 1,138 patients were admitted to the cardiac service during the period of recruitment. Of these, 927 received diagnoses other than Type 1 MI, with the remainder (211) having STEMI or NSTEMI. Most of these patients (76.3%) did not satisfy inclusion/exclusion criteria and 12 patients (7.1%) refused to be involved. Seven (7) (4.2%) of those excluded were unable to be enrolled on the basis of clinical instability.

Baseline Characteristics

A total of 43 patients were enrolled into this study, with STEMI and NSTEMI receiving approximately equal representation (21 and 22, respectively). The mean patient age was 59.9 years and almost three-quarters of patients were male (Table 1). Patients were of high cardiovascular risk, with a mean body mass index of 30.0, 65.1% having hypertension, 58.1% having dyslipidaemia and 27.9% having diabetes.

Prior to presentation, 27.9% of patients had preceding symptoms of angina, and two patients were in atrial fibrillation. 25.6% of patients had a history of MI, with eight of these patients receiving subsequent percutaneous coronary intervention (PCI). No patient had a history of cardiac surgery, rheumatic heart disease (RHD), myocarditis or infective endocarditis (IE).

The mean time from symptom onset to hospital presentation was 4.3 hours. Upon arrival to hospital, over 10% of patients had pulmonary oedema and one had an episode of ventricular tachycardia. Most patients had good premorbid cardiac function, with 83.7% being asymptomatic (New York Heart Association (NYHA) Class I) at baseline. Among the STEMI subgroup, eight patients (38.1%) were thrombolysed.

The mean troponin I on admission was 0.8 μg/L (<0.04 μg/L), while the mean peak troponin was 24.5 μg/L. Most patients had preserved systolic cardiac function, with a mean ejection fraction (EF) of 53.7% (±11.6). Angiographic assessment revealed that left anterior descending (LAD) artery lesions were the most common source of culprit obstruction (44.1%), followed by right coronary artery (RCA) (35.3%) and circumflex (16.3%) (Table 1).

At study completion, 10 patients were incompletely followed up. Five (5) of these did not return for any follow-up, while the remainder missed one of the two time points.

Anti-Myosin Antibody Levels

In total, 32 patients (74.4%) were positive for IgM on admission. The majority of these patients (71.9%) remained positive at both follow-up time points. Five (5) patients (11.63%) were negative at admission, but seroconverted during follow-up. In patients with STEMI and NSTEMI, a mean reduction of 3.54% (±0.2) in IgM was observed between admission and first follow-up, and 6.04% (±0.1) by the conclusion of the study.

There was no difference in mean IgM ($p = 0.9$, $p = 0.07$), or the proportion of antibody positive patients ($p = 0.76$, $p = 0.92$), between the STEMI and NSTEMI cohorts at either follow-up time-point (Figure 1 and Table 2, respectively).

Anti-myosin antibody IgM positive and negative patients did not differ with respect to cardiovascular history, or risk factors, and both had similar demographic profiles. There was no observed difference between time to hospitalisation, peak cTnI, CK-MB or BNP between groups. However, there was a moderate positive correlation between peak cTnI and the percentage increase in IgM level at first follow-up, ($r(98) = 0.455$, $p = 0.015$). This was mirrored by a positive correlation, of similar magnitude ($r(98) = 0.456$, $p = 0.015$), between cTnI area under the curve and percentage increase in IgM at first follow-up.

Among the STEMI cohort, all thrombolysed patients were positive for IgM. Although these patients accounted for 50% of all antibody positive participants, this observation did not reach statistical significance ($p = 0.11$).

On admission, 10 (23.3%) participants were positive for anti-myosin IgG. Of these, six (60.0%) remained positive at each of the two follow-up time points. The remaining four

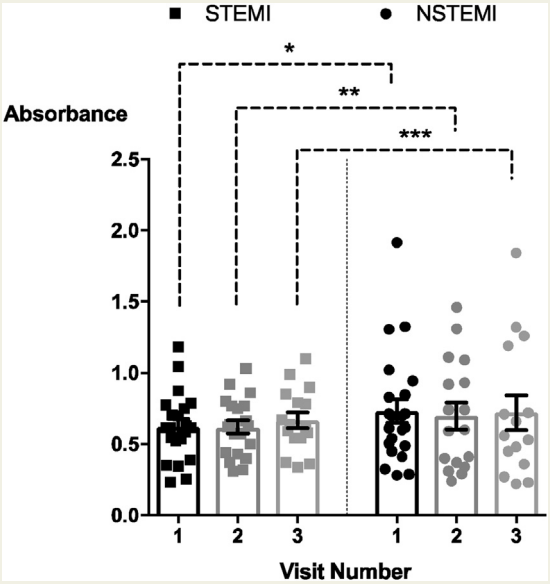

Figure 1 Comparison of IgM antibodies between STEMI and NSTEMI patients.
Comparison of IgM levels between STEMI and NSTEMI participants, with mean and standard error values. *p = 0.576, ** p = 0.910, ***p = 0.066.

Table 2 Comparison of antibody positivity between STEMI and NSTEMI patients at each time point.

	IgM			IgG		
	Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
STEMI [n (%)]	16 (76.2)	15 (75.0)	15 (75.0)	1 (4.8)	4 (21.1)	2 (16.7)
NSTEMI [n (%)]	16 (72.7)	12 (70.6)	11 (73.3)	9 (40.9)	9 (52.9)	9 (64.3)
P-value	0.795	0.763	0.916	0.009	0.047	0.014

Abbreviations: STEMI, ST-Elevation myocardial infarction; NSTEMI, non-ST-Elevation myocardial infarction; IgM, immunoglobulin M; IgG, immunoglobulin G.

patients (40.0%), who were negative at first follow-up, became positive at the conclusion of the study. Unlike IgM, the mean IgG increased between admission and follow-up, by 1.24% (±0.283) at time point one, and 13.55% (±0.132) at time point two.

Non-STEMI patients were much more likely to be positive for anti-myosin IgG than those in the STEMI cohort ($p = 0.009$) at admission. This was accompanied by a significant difference in mean antibody levels between the two groups at the same time point ($p < 0.0001$). These differences persisted throughout follow-up (Figure 2).

We did not observe any significant difference in demographic variables, cardiovascular history or risk factor profiles between antibody positive and negative patients. However, patients with a history of MI trended towards being more likely to be antibody positive at admission ($p = 0.09$).

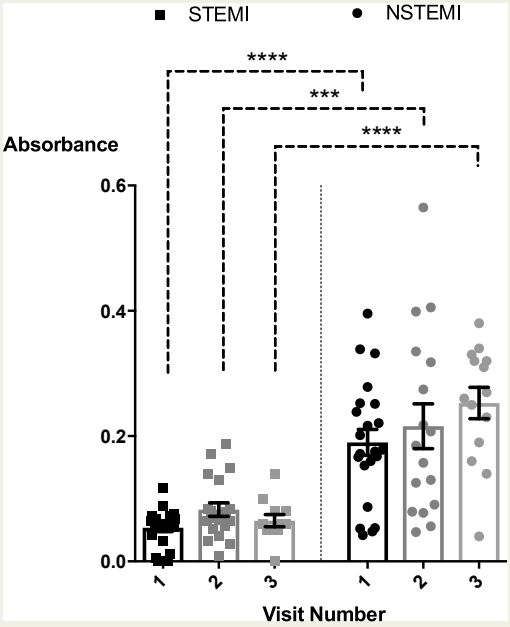

Figure 2 Comparison of IgG antibodies between STEMI and NSTEMI patients.
Comparison of IgG levels between STEMI and NSTEMI participants, with mean and standard error values. ***$p < 0.001$, **** $p < 0.0001$.
Abbreviations: STEMI, ST-Elevation myocardial infarction; NSTEMI, non-ST-Elevation myocardial infarction; IgG, immunoglobulin G.

Antibody Kinetics

The mean change in anti-myosin IgM and IgG, between admission and first follow-up, was similar between groups ($p = 0.31$ and $p = 0.69$ respectively). However, at second follow-up, NSTEMI patients had a mean reduction in IgM optical density (-0.12 ± 0.14), whereas STEMI patients had a mean increase (0.01 ± 0.13) ($p = 0.032$). This relationship was also observed for the IgG isotype, with STEMI patients having a mean increase of 27.47% in antibody signal (±0.53) compared to a mean reduction of 16.67% in NSTEMI patients (±0.36) ($p = 0.041$) (Figure 3).

Functional Significance

The formation of AMA, of either isotype, was not associated with an unfavourable outcome at 6 months of follow-up. There was no difference in NYHA functional class, BNP level, echocardiographic findings, recurrent MI or mortality in antibody positive and negative patients. At 6 months of follow-up, AMA IgG negative patients tended to have a greater median reduction in BNP between admission and follow-up (46.50 μg/L), compared with their IgG positive counterparts (21.50 μg/L), although this observation didn't reach statistical significance ($U = 53$, $z = -1.53$, $p = 0.126$)

Discussion

There are few studies investigating the formation and functional significance of anti-myosin IgM in MI patients. The observation that 74.4% of MI patients are positive for anti-myosin IgM at admission is consistent with the values reported by De Scheerder et al. [21], and suggests that this process may be rapidly induced in response to cardiomyocyte necrosis. This response appears to abate in ensuing months, with antibody concentration reducing more rapidly in NSTEMI patients. In the context of a moderately positive association observed between cTnI and mean change in IgM concentration at follow-up, this finding is likely related to the larger infarctions experienced by the STEMI cohort, which leads to a greater antigenic stimulus for antibody formation.

Although not statistically significant, the observation that all thrombolysed patients were positive for anti-myosin IgM

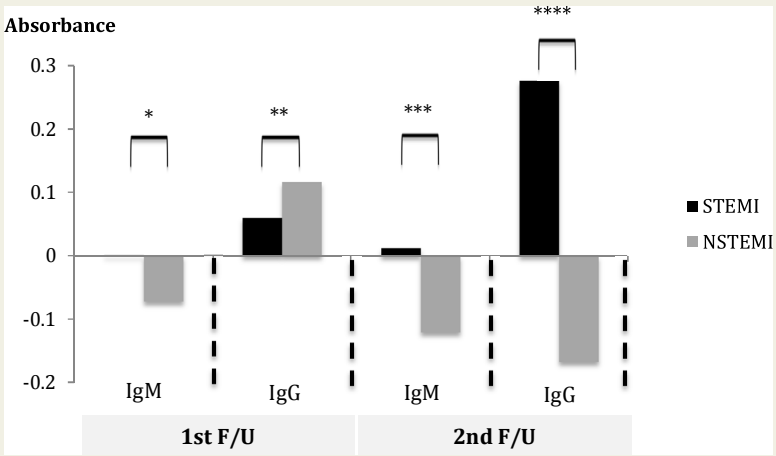

Figure 3 Mean change in IgM and IgG levels between baseline and follow-up.
The change in antibody signal for IgM and IgG was determined for each sample and expressed as a percentage of the baseline value. The mean change is presented here for each patient cohort and antibody isotype. *p = 0.31, **p = 0.69, ***p = 0.032, ****p = 0.041.
Abbreviations: STEMI, ST-Elevation myocardial infarction; NSTEMI, non-ST-Elevation myocardial infarction; IgM, immunoglobulin M; IgG, immunoglobulin G.

is interesting, particularly in the context of emerging evidence implicating plasmin in increased cytokine production, leucocyte chemotaxis and inflammation. [31,32] The relationship between activation of the fibrinolytic pathway, and post-infarction autoimmune responses is worthy of further investigation.

Given that secondary immune responses result in IgG formation, the observation that IgM positivity and concentration were unaffected by previous myosin exposure, through MI or PCI, is not surprising. Similarly, although anti-myosin IgM are known to infiltrate the inflamed myocardium [33], they are short-lived [34,35], have high avidity [36] and are often weakly cross-reactive [37]. These characteristics may limit the potential for IgM to contribute toward long-term myocardial damage and dysfunction. This is consistent with our observation that anti-myosin IgM positivity did not affect patient symptomatology, BNP level or echocardiographic findings at follow-up.

Our results have both confirmed and extended the findings of previous work about anti-myosin IgG in patients with MI. We report that 23.3% of patients were positive for IgG at admission, which is consistent with observations made by Pang (27%) [11], Elahi (26%) [38], and De Scheerder in two separate studies (16% and 35% respectively) [21,39,40]. However, for the first time, we have stratified participants according to MI type, and found that NSTEMI patients were significantly more likely to be positive for IgG AMA at baseline. This may be related to the fact that some patients with NSTEMI have a more subacute presentation than their STEMI counterparts.

The relationship between infarct size and anti-myosin IgG has been divisive. Some have argued that a dose response relationship exists between cardiac biomarker concentration and IgG response, against both myosin [10] and troponin [41,42]. Others have proposed that intracellular protein leakage is of little consequence in determining the magnitude of a subsequent antibody response [20]. Although a larger increase in mean IgG signal was observed in STEMI patients, this was independent of troponin. Therefore, our results support the latter hypothesis, with cTnI having little effect on AMA formation or concentration.

The association between BNP, which is a marker of ventricular strain, and anti-myosin IgM or IgG antibody formation has not been previously investigated in the context of MI. However, Leuschner et al. have reported no difference in mean BNP between anti-troponin IgG positive and negative patients [43]. Our results are consistent with this finding, with median BNP having no relationship with the formation of either antibody isotype, at any time point.

Recurrent antigenic exposure is associated with the activation of T-helper cells, which usually leads to a robust IgG response [44–46]. Consequently, it is plausible that patients with preceding myocardial damage, through any cause, mount a greater IgG response following MI than those without a history of cardiac disease. This hypothesis has not been previously investigated in the context of AMAs. However, surprisingly, Lindahl et al. reported that a history of cardiac disease was not associated with a greater risk of developing anti-troponin antibodies [41]. Our results are consistent with this observation, with previous MI, PCI and cardiac surgery occurring with similar frequency in AMA positive and negative patients.

The observation that AMA positivity was not associated with an unfavourable NYHA classification is important. However, it does not preclude the possibility that these antibodies contribute to symptoms of cardiac insufficiency after MI, for two reasons. Firstly, NYHA is a relatively blunt tool to detect differences in

cardiac function and can be confounded by pathology of a number of organ systems. Secondly, although mitigated through the use of a standardised questionnaire, the NYHA assessment is inherently subjective. Non-differential biases, such as this, tend to favour the null hypothesis. There are, therefore, two main conclusions that can be drawn from our findings. The first is that AMAs do not affect patient symptomatology. Alternatively, it is also possible that AMA formation is related to outcome, and that our results are explained by the fact that NYHA criteria are insensitive to their effect, or that pathogenic effects are not appreciable at 6 months post MI.

Echocardiographic variables have shown the most promise as suitable endpoints to detect the functional effect of anti-cardiac antibodies. In a cohort of MI patients, Leuschner et al. reported that anti-troponin antibody negative patients had a greater left ventricular ejection fraction and stroke volume than antibody positive participants at 6–9 months of follow-up [43]. These findings have not been reproduced in the context of AMA, in either the present study, or by Pang et al. [11] Given that both of these studies had less than 70 participants, neither exclude an echocardiographically appreciable effect of AMA on cardiac recovery, and further research in this area is suggested.

Although the difference was not statistically significant, the observation that anti-myosin IgG negative patients had a greater median reduction in BNP than their IgG positive counterparts, is suggestive of the possibility that AMA have a restraining effect on cardiac recovery in MI patients. However, this proposal requires clarification in larger studies.

Our observation that AMA were not associated with recurrent MI or elevated mean cTnI at follow-up was incongruent with that reported by Dangas et al., in their cohort of 33 patients who were followed up for 6 months [10]. Given that the sample size and duration of follow-up were greater in the present study, this is unlikely related to power or follow-up duration. However, current mechanistic data do not support a potential role of AMA in producing recurrent myocardial ischaemia and, in this context, our findings were not surprising.

The demographic and clinical characteristics of included participants are consistent with those reported in the national [47] and international [48] MI literature. These patients were managed with a standard of care that reflects modern cardiac practice. As such, our results are likely to be externally valid and, therefore, applicable to cohorts of MI patients receiving care in modern, well-resourced hospitals. Similarly, the risk of selection bias was reduced by consecutive enrolment of patients that were eligible for inclusion in our study and the fact that losses to follow-up were minimal. Compared with other relevant literature, our study had a relatively large sample size, second only to that conducted by De Scheerder et al., with 80 patients, over 25 years ago [21].

This study had some limitations. Firstly, it comprised a small population of MI patients, who were enrolled from a single centre. These participants were followed up for 6 months following their index event. It is possible that the effects of AMA become more apparent after this time-point. Moreover, echocardiographic variables, NYHA classification and BNP are potentially insensitive endpoints by which to measure the functional significance of AMA, particularly within the first 6 months of MI. For these reasons, the presented results are hypothesis generating, and require further clarification in larger studies with longer follow-up.

A significant proportion of patients generate anti-myosin IgM and IgG following MI. These antibodies have been causally associated with myocardial damage and dysfunction in animal models of cardiac failure. Surprisingly, this study revealed that patients with subendocardial MI had much higher admission AMA levels, and were more likely to be positive for anti-myosin IgM than those with transmural infarctions. However, NSTEMI patients had reductions in IgM and IgG levels over 6 months of follow-up, compared with an increase in both isotypes at follow-up among those with STEMI. Future research should aim to further characterise the MI patients at risk of AMA formation, the functional significance of these antibodies and the specific epitopes that they target. This may result in the emergence of novel therapies to attenuate cardiac dysfunction and improve outcome in patients with MI.

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