SCIENTIFIC LETTER

Reduced post-infarction myocardial apoptosis in women: a clue to their different clinical course?

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Heart failure (HF) is less prevalent and has a better prognosis in women than in men. Also, the outlook after acute myocardial infarction (AMI) is more favourable in elderly women.¹ Such sex related differences are probably related to sex specific cardiac remodelling processes.² However, despite several hypothetical pathogenetic mechanisms, there are no established explanations.

Myocardial apoptosis is a pivotal determinant of left ventricular (LV) dysfunction and cardiac failure in experimental and clinical studies, including after AMI. Sex may indeed influence apoptosis, as shown in normal aging and in end stage HF.³ However, little is known about the role of sex in post-infarction apoptosis. We thus evaluated the influence of sex on expression of pro-apoptotic mediators and myocardio-cyte apoptotic index (AI) in subjects dying after AMI.

METHODS

Using established methods,⁴ we selected six females who had died after AMI with permanent infarct related artery occlusion at necropsy (< 30 hours after death); 15 males with similar characteristics were selected during the same period. Three men and one woman dying from non-cardiac causes were included as controls.

Re-infarction was excluded on clinicopathologic grounds in all cases. Infarct size was quantified at gross pathology on a four grade scale (small, moderate, moderate to extensive, and extensive).

Tissue specimens were obtained at peri-infarct and remote sites. In situ end labelling of DNA fragmentation by transferase-mediated biotinylated UTP nick end labelling (TUNEL) was performed (Apoptag, Oncor) and sections were stained with antibodies against muscle actin (DAKO-Carpintera; dilution 1:50), and activated caspase-3 (Cell Signaling Technology; dilution 1:50). Mycardiocytes were defined as apoptotic by co-localisation of TUNEL and caspase-3. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) (DAKO; dilution 1:100) and SC-35 (Sigma; dilution 1:200) was also performed. AI was defined as the ratio of mycardiocytes co-expressing TUNEL and caspase-3 positivity on nucleated cells per field ($\times 250$), calculated on 100 random fields. Muscle actin negative cells, TUNEL positive but PCNA or SC-35 positive cells were not included in cell counts. Immunohistochemistry for myocardial expression of the pro-apoptotic mitochondrial protein bax was performed in 14 cases (four females, 10 males; Santa Cruz; dilution 1:100). Immunoreactivity was quantified as percentage of positive mycardiocytes per field ($\times 250$) in an average of 50 fields. Immunohistochemical staining for the expression of cyclooxygenase-2 (COX-2), a potential marker of subacute myocardial ischaemia, was performed in four cases using a primary anti-COX-2 antibody (Santa Cruz; dilution 1:100). Results were described on a dichotomic (positive/negative) scale.

SPSS 10.1 was used as indicated for χ^2 (Fisher corrected where appropriate), Mann-Whitney, Wilcoxon,

Kruskal-Wallis, and Spearman tests, with two tailed significance at 0.05, were also used. Multivariable analysis was performed using a generalised linear model including univariate predictors of AI at the 0.10 level.

RESULTS

Demographic, clinical, and pathologic characteristics were not different between males and females (table 1).

Myocardial AI in peri-infarct regions correlated significantly with parameters of unfavourable LV remodelling (LV thickness, $R = -0.48$, $p = 0.029$, diameter to thickness ratio, $R = +0.56$, $p = 0.008$). Comparing specific patterns of remodelling (from compensatory hypertrophy to eccentric hypertrophy to overt dilatation), AI appeared lower in subjects with favourable LV remodelling, and significantly higher in those with eccentric hypertrophy or overt dilation ($p = 0.046$).

Myocardial AI in peri-infarct myocardium was 10-fold higher in men than in women, 25.9% (17.9–28.6) v 2.6% (0.5–14.1), $p = 0.003$ (fig 1A). A non-significant trend towards a twofold increase in AI in men ($p = 0.066$) was found at remote sites (fig 1B). Moreover, AI was significantly higher at peri-infarct versus remote regions in the overall population ($p < 0.001$), as well as in males ($p = 0.001$) and females only ($p = 0.027$). Apoptotic mycardiocytes were extremely rare in control hearts (0.01%, 0.01–0.02%), with no sex differences.

A higher number of mycardiocytes expressing cytoplasmic bax immunostaining was found in peri-infarct regions in males versus females, 55% (44–61) v 14% (2.5–42), $p = 0.012$ (fig 1C). A non-significant trend was also present in remote areas, 2.7% (0.8–4.1) v 0.9% (0.4–3.0), $p = 0.23$ (fig 1D). Moreover, bax expression at the peri-infarct site was significantly associated with macroscopic signs of unfavourable LV remodelling such as thickness ($R = -0.68$, $p = 0.008$), and diameter to thickness ratio ($R = +0.82$, $p < 0.001$) (fig 1E). The link between myocardial apoptosis, bax expression, and ischaemia was further strengthened by the finding of intense immunostaining for COX-2 myocardial expression in peri-infarct regions in subjects with higher than median myocardial apoptosis and bax staining (fig 2) (to see fig 2 visit the Heart website—<http://www.heartjnl.com/supplement>).

Multivariable analysis, assessing the independent role of the four borderline univariate predictors of increased peri-infarct AI (age, sex, HF, and trauma as cause of death), showed maleness to be a significant predictor of AI ($p = 0.039$).

Abbreviations: AMI, acute myocardial infarction; AI, apoptotic index; COX-2, cyclooxygenase-2; HF, heart failure; LV, left ventricular; PCNA, proliferating cell nuclear antigen; TUNEL, transferase mediated biotinylated UTP nick end labelling.

Table 1 Clinical and pathologic characteristics

	Females (n=6)	Males (n=15)
Clinical characteristics		
Age (years)	79 (73–85)	72 (65–79)
Previous remote AMI (>6 months) (%)	2 (33%)	9 (60%)
Time from AMI to death (days)	15 (12–24)	25 (12–50)
Fibrinolytic treatment (%)	4 (66%)	9 (60%)
Symptomatic heart failure (%)	2 (33%)	11 (73%)
Trauma as cause of death (%)	0	4 (27%)
Pathologic characteristics		
Anterior AMI (%)	3 (50%)	5 (33%)
Transmural AMI (%)	6 (100%)	13 (87%)
*Large AMI (%)	6 (100%)	10 (66%)
Cardiac weight (g)	500 (475–605)	510 (460–600)
Hamber diameter/wall thickness ratio	8.6 (6.4–10.9)	10.4 (8.7–12.7)

*Defined as an area of transmural necrosis involving more than one LV wall (approximately 30% of the circumference, or moderate to extensive, or extensive infarct) at pathology.
Data are expressed as median (interquartile range), unless otherwise stated; no significant differences were found between the two groups.

DISCUSSION

To the best of our knowledge, the present study shows for the first time that in subjects dying after AMI peri-infarct apoptosis is greater in men than women. In this clinicopathologic

model, males also show increased post-infarction myocardial expression of bax in comparison to females.

HF is more frequent and severe in men than in women.¹ Sex related differences in cardiac remodelling are probably among the explanations of such sex differences in clinical outcomes.² However, underlying mechanisms are still unclear, but may include the actions of oestrogens, telomerase, Akt, sodium/calcium exchanger, β adrenergic receptors, and the renin–angiotensin system.

Similarly to findings by Guerra and colleagues,³ we show sex specific differences in AI after AMI. We can thus hypothesise that males and females may have a different modulation of the apoptotic pathway in the peri-infarct region; females could be partially protected from ischaemia induced bax expression and pro-apoptotic activity in comparison to men, similar to findings in cardiac fibroblasts.⁵

A cause–effect link between sex, apoptosis, and LV remodelling cannot however be demonstrated by the present observational data, and great caution should be applied when extrapolating our results to clinical practice as the study was limited by the small sample size and the non-experimental design.

In conclusion, myocardial apoptosis in peri-infarct areas is higher in males dying late after AMI than in females, and this difference is associated with increased myocardial bax expression. These findings may explain the more aggressive course of post-infarction HF in men and the relatively more

Figure 1 Sex and post-infarction apoptosis and bax expression in peri-infarct and remote myocardial region; (A) males (open columns) have a greater apoptotic index (AI) than females (solid columns) in peri-infarct region ($p = 0.003$); (B) men also have higher AI than women in remote myocardial region ($p = 0.066$); (C) males ($n = 10$, open columns) have greater bax expression than females ($n = 4$, solid columns) in infarct regions ($p = 0.012$) and (D) higher expression in remote myocardial regions ($p = 0.23$); (E) myocardial expression of bax in the infarct myocardium and signs of post-infarction LV remodelling expressed as transverse diameter to wall thickness ratio ($R = +0.82$, $p < 0.001$). Males are shown as open diamonds, females as solid diamond. Column heights represent median values, whiskers represent interquartile ranges.

benign post-infarction remodelling in women, potentially suggesting an increased resistance to ischaemia in females. The recognition and thorough characterisation of the influences of sex on cardiac disease may indeed provide keys to cardiovascular pathophysiology, which may eventually benefit both sexes.

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Dr G Biondi-Zoccai presented this study as an abstract at the 2002 Italian Society of Cardiology Congress winning a Young Investigator Award.

To view fig 2 please visit the Heart website—<http://www.heartjnl.com/supplemental>

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IMAGES IN CARDIOLOGY

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Complete aortic dissection demonstrated by intraoperative transoesophageal echocardiography

A 75 year old man with a history of hypertension and coronary artery disease presented with syncope following a cardiac arrest at home. He was sent to our hospital from another centre with a diagnosis of aortic dissection. The patient was unconscious, intubated, and his overall condition was poor. He was sent to the operating room immediately where he suffered a severe hypotensive episode. Transoesophageal echocardiogram (TOE) revealed a linear structure going up and down from the aorta to the left ventricle. In the two chamber view at 90° this structure appeared like a tube extending into the left ventricle (below left). In the left ventricular outflow track view at 120° the complete dissection of the aorta from the aortic arch to the sinotubular junction was observed (below right). The dissection produced a severe aortic regurgitation with

dilatation of the left ventricle. We performed a complete repair of the aortic arch with a supracoronary tube. The subsequent echocardiogram revealed only mild aortic regurgitation. However, the patient died five days later because of brain damage caused by the cardiac arrest.

A complete dissection of the aorta is not very common. In this case, the dissection was spectacular due to the complete separation of the intima, which produced these images on TOE.

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SCIENTIFIC LETTER

Prognostic impact of new onset atrial fibrillation in acute non-ST elevation myocardial infarction data from the RICO survey

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New onset atrial fibrillation (AF) is a common complication of acute myocardial infarction (MI), with a prevalence ranging of from 7–18%, and is associated with a higher incidence of in-hospital congestive heart failure. AF occurs in patients who are older with severe coronary artery disease and is associated with higher 30 day and one year mortality rates than for those patients without AF.^{1–4}

However, most studies have been performed on data collected from patients with ST elevation myocardial infarction (STEMI). In the present study, we examined the characteristics and in-hospital outcome for a non-selected population, hospitalised for non-ST elevation myocardial infarction (NSTEMI) and included in the French regional RICO survey.

METHODS

From 1 January 2001 to 31 July 2003 pre-hospital as well as in-hospital data from 504 patients, hospitalised for acute NSTEMI in one region of eastern France, were analysed. All the cardiology departments of the region, five public hospitals and one private clinic, in charge of cardiac emergencies, participated in the study.

All patients included in the study presented with NSTEMI. The diagnoses were based on: an increase in troponin concentrations to the upper limit of normal (ULN) or creatine kinase myocardial band ≥ 2 ULN; NSTEMI (ST segment depression or negative T wave on ECG); patients admitted to index hospital within 24 hours following symptom onset. Patients presenting any one of the following criteria were excluded from the study: persistent ST elevation or new Q waves on ECG; unconfirmed MI diagnosis on behalf of another diagnosis (pulmonary embolism, aortic dissection, acute pericarditis); post-percutaneous transluminal coronary angioplasty MI or post-coronary artery bypass graft MI; delay from symptom onset to presentation > 24 hours; permanent AF before admission.

Data collection was prospective, uniform, and standardised in all centres. Medical data for each patient were collected from the moment the mobile emergency unit took charge of the patient (time zero) to discharge. Demographic data and cardiovascular risk factors were collected from time zero; clinical data at hospital admission, the main therapeutic agents administered during the first 48 hours, and the main cardiac events during hospitalisation (from time zero to discharge) were also recorded. Left ventricular ejection fraction (LVEF) was evaluated for 372 (74%) patients either by contrast or radionuclide ventriculography or echocardiography.

New onset AF was defined when AF developed < 24 hours after MI onset. According to recent guidelines,⁵ ECGs were monitored for the occurrence of AF or ventricular

arrhythmias including ventricular tachycardias (recording of more than three ventricular ectopic beats) and ventricular fibrillations, for three days after admission. Holter electrographic tracings were collected for 24 hours before discharge.

Two groups of patients were compared: the AF group included patients who developed AF < 24 hours after MI onset, and the sinus rhythm (SR) group included all other patients with SR. Results were expressed as median time (25th–75th centile). Qualitative data were compared using a χ^2 test modified by Yates. Quantitative parameters were compared by the Student unpaired *t* test. A multiple logistic regression model was chosen to assess the relation between variables and the occurrence of events. Model building involved selecting the variables that were prognostic for adverse hospital outcome in multiple regression analyses. The first model was built with variables that are known predictors of AF and that were predictors in univariate analysis (age, Killip class > 2 , chronic obstructive pulmonary disease (COPD), smoking, and hypertension). The second model tested AF after adjustment for potential confounding factor (age, Killip class > 2 , cardiogenic shock, and LVEF) as a predictor for death or ventricular arrhythmia. Age and LVEF has been dichotomised according to classical data from the literature (age > 75 years and LVEF $< 45\%$). The significance level required to be entered in multivariate analysis was 10%. The Wald test was performed to test for significance. Results are expressed as odds ratio (OR) with 95% confidence intervals (CI). All tests were two sided. Differences were significant at the 5% level ($p < 0.05$).

RESULTS

Of the 504 NSTEMI patients included in the study, 39 (7.6%) were in the AF group and 465 (92.4%) were in the SR group.

Age was higher in the AF versus the SR group (77 (73–83) years old *v* 70 (56–79) years old, respectively, $p < 0.001$). The sex ratio was similar for the two groups. On admission, the percentage of patients with clinical heart failure (Killip > 2) was higher in the AF group compared to the SR group (36% *v* 18% respectively, $p = 0.014$).

With regard to mean heart rate before AF onset, patients with AF had increased heart rate on admission (97 (85–130) bpm *v* 76 (65–90) bpm, $p = 0.0003$). No differences were observed between the two groups for primary revascularisation procedures.

Abbreviations: AF, atrial fibrillation; COPD, chronic obstructive pulmonary disease; GUSTO-I, global use of streptokinase and t-PA for occluded coronary arteries; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; SR, sinus rhythm; STEMI, ST elevation myocardial infarction; ULN, upper limit of normal

Table 1 Cardiovascular events, mortality rate, and duration of in-hospital stay

	SR group (n=465)	AF group (n=39)	p Value
Death	29 (6%)	8 (21%)	0.003
Death or VT/VF	48 (10%)	11 (28%)	<0.001
VT/VF	27 (6%)	4 (10%)	0.445
Cardiogenic shock	33 (7%)	6 (15%)	0.121
MI recurrence	53 (11%)	7 (18%)	0.339
Duration of stay (days)	4 (3–6)	4 (3–7)	0.696

Data are presented as percentages or median (25th–75th centile).
MI, myocardial infarction; VF, ventricular fibrillation; VT, ventricular tachycardia.

In a multivariate model, when adjusting for age > 75 years, Killip class > 2, COPD, not smoking, and hypertension, only age remained associated with the occurrence of AF ($p = 0.038$).

In-hospital mortality and the combined criterion (in-hospital mortality or ventricular tachycardia/ventricular fibrillation) was higher in the AF group than in the SR group (21% and 28% in the AF group *v* 6% and 10% in the SR group, $p = 0.003$ and $p < 0.001$, respectively) (table 1).

After adjustment for significant predictors of mortality (age, Killip class > 2, cardiogenic shock, and LVEF < 45%), AF remained an independent predictor for cardiac death or ventricular arrhythmia (OR = 2.23; $p < 0.001$).

DISCUSSION

The main findings of this study are: firstly, AF occurred in 7.7% of an unselected NSTEMI population; secondly, an age of > 75 years is strongly associated with a risk of AF in this population; thirdly, AF is an independent predictor for cardiac death and/or ventricular arrhythmia in patients with NSTEMI.

Higher Killip class at admission, observed in the AF group, suggests that haemodynamic compromise is the most likely mechanism of this rhythm disturbance, as described in previous studies including STEMI patients.³ The occurrence of congestive heart failure was significantly higher in the AF group than in the SR group. This finding may be because of acute worsening of cardiac haemodynamic variables from the loss of atrial contraction.

In the present study, the mortality rate in NSTEMI patients with AF was higher than that reported in the GUSTO-I

experience (21% *v* 13.8%, respectively).² This finding is partly explained by the differences in therapeutic management (reperfusion strategy) between the two series—all STEMI patients enrolled in GUSTO-I were thrombolytic eligible, while only 11% benefited from a percutaneous coronary intervention in our study, as recommended for patients judged to be at high risk for MI or death.³ In addition, in the NSTEMI population, the occurrence of AF seems to be associated with a worse in-hospital prognosis than in the STEMI population.

In summary, this is the first study that has examined the incidence and prognostic implications of AF in NSTEMI patients. AF is not an infrequent event during NSTEMI and is an independent predictor for cardiac death or ventricular arrhythmia in these patients.

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CORRECTION

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The following authors names were misspelt in the credits to this article: M-P Di Marino (not M-P D Marino), F De Giorgio (not F D Giorgio), and A Abbate (not A Abate).