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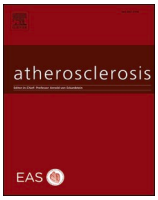

# Association of autoantibodies targeting endothelin type-A receptors with no-reflow in ST-elevation myocardial infarction

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A B S T R A C T

*Background and aims:* No-reflow (NR), where the coronary artery is patent after treatment of ST-elevation myocardial infarction (STEMI) but tissue perfusion is not restored, is associated with worse outcomes. We aimed to investigate the relationship between autoantibodies activating endothelin-1 receptor type A (ETAR-AAs) and NR after primary percutaneous coronary intervention (PPCI) in STEMI.

*Methods:* We studied 50 patients (age 59 ± 11 years, 40 males) with STEMI who underwent PPCI within 6 h after the onset of symptoms. Blood samples were obtained from all patients within 12 h following PPCI for ETAR-AA level measurement. The seropositive threshold was provided by the manufacturer (>10 U/ml). NR was assessed by cardiac magnetic resonance imaging (MVO, microvascular obstruction). As a control group, 40 healthy subjects matched for age and sex were recruited from the general population.

*Results:* MVO was observed in 24 patients (48%). The prevalence of MVO was higher in patients with ETAR-AAs seropositivity (72% *vs.* 38%, $p = 0.03$). ETAR-AAs were higher in patients with MVO (8.9 U/mL (interquartile range [IQR] 6.8–16.2 U/mL) *vs.* 5.7 U/mL [IQR 4.3–7.7 U/mL], $p = 0.003$). ETAR-AAs seropositivity was independently associated with MVO (OR 3.2, 95% CI 1.3–7.1; $p = 0.03$). We identified ≥6.74 U/mL as the best cut-off for prediction of MVO (sensitivity 79%; specificity 65%; NPV 71%; PPV 74%; accuracy 72%).

*Conclusions:* The ETAR-AAs seropositivity is associated with NR in STEMI patients. These findings may open up new options in the management of myocardial infarction even if confirmation in a larger trial is needed.

## 1. Introduction

Acute myocardial infarction is one of the leading causes of death and morbidity in Western countries [1]. Primary percutaneous coronary intervention (PPCI) by transluminal balloon angioplasty and stent implantation has become the method of choice for the treatment of ST-segment elevation myocardial infarction (STEMI) [2]. Nevertheless, in many cases, myocardial damage is not immediately terminated after elimination of epicardial occlusion with successful PPCI. The combination of ischaemic damage and reperfusion injury may prevent the restoration of myocardial perfusion despite good epicardial coronary artery flow. This phenomenon is termed myocardial no-reflow (NR) and is associated with extensive tissue necrosis, infarct expansion, congestive heart failure, and death [3].

The pathophysiology of NR is not completely understood. Several mechanisms have been identified in experimental models, including extravascular compression, microvascular vasoconstriction, and platelet–leukocyte capillary plugging [3]. Reperfusion injury, which may lead to the NR phenomenon, is a multifactorial process that focuses on coronary microcirculation [4]. The coronary endothelium is the most important regulator at this level. The release of reactive oxygen species by neutrophils and proinflammatory mediators that can directly cause endothelial damage has been described in NR [5,6].

Endothelin-1 receptor type A (ETAR) is a G-protein-coupled receptor (GPCR) expressed on the surface of a great variety of cells: endothelial cells, vascular smooth muscle cells, immune cells, and fibroblasts

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express ETAR, which is activated by endothelin-1 [7]. Autoantibodies specific for ETAR (ETAR-AAs) can bind these receptors and regulate their function [8]. The function of ETAR-AAs is similar to that of natural ligand, and it involves not only vasoconstriction but also the secretion of proinflammatory cytokines, collagen production by fibroblasts, and reactive oxygen species release by fibroblasts and neutrophils [9]. Interestingly, these features are also described in NR [3]. As NR is associated with adverse clinical consequences, it is of great importance to identify the risk factors, the exact underlying mechanisms, and adopt effective preventive and therapeutic strategies.

ETAR-AAs seropositivity has been detected in healthy volunteers who served as a control group in many studies [10]. Moreover, in patients with autoimmune connective tissue diseases, ETAR-AAs seropositivity increases the risk of future development of vasculopathy [11]. In the literature, there are no data regarding the relationship between NR and the presence of autoantibodies. Therefore, we hypothesized that after reopening of the epicardial coronary artery, the preexistence of ETAR-AAs might have detrimental effects on coronary microcirculation, resulting in microvascular obstruction (MVO) and, thus, NR.

## 2. Patients and methods

### 2.1. Study population

This prospective study was conducted at Padua University Hospital between January 2022 and June 2022. The study protocol was approved by the local ethics committee (code number CESC 5478/AO/22), and all patients gave written informed consent. Patients were eligible for study participation if they presented with chest pain (>30 min) and persistent ST-segment elevation of 0.2 mV in two or more adjacent leads on standard ECG, within 6 h of symptom onset and either an occluded culprit artery (TIMI coronary flow grade ≤1) or reduced flow (TIMI flow grade 2, slow but complete filling) in the presence of angiographic evidence of thrombus (TIMI thrombus grade ≥2). Eligibility required occlusion in the proximal or mid segment of a major coronary artery. Exclusion criteria included a functional coronary collateral supply (Rentrop grade ≥2) to the culprit artery, previous myocardial infarction and contraindications to cardiac magnetic resonance (CMR) at study enrolment.

As a control group, 40 healthy subjects matched for age and sex were recruited from the general population (Hospital staff and healthy blood donors). In particular, all healthy subjects were asymptomatic with no history of heart disease and endocrine disease. Exclusion criteria for all subjects included any of the following conditions: cerebral vascular disease, carotid artery bruit, peripheral bruit or abnormal pulse, history of angina or myocardial infarction, hypertension requiring treatment. All participants had normal ECG at rest. Patients and healthy subjects came from the same geographic area (northeast Italy). In healthy subjects, the absence of coronary artery disease was evaluated by clinical history, physical examination, and ECG.

### 2.2. PPCI procedure

All patients received 250 mg of aspirin and heparin (60 U/kg body weight) intravenously before PPCI. Prasugrel (started at a loading dose of 60 mg and continued with a maintenance dose of 10 mg once daily) or ticagrelor (started with a loading dose of 180 mg and continued with a maintenance dose of 90 mg twice daily) administration was mandatory. Aspirin was given indefinitely at a dose of 100 mg/day. The use of glycoprotein IIb/IIIa inhibitors, angiotensin-converting enzyme inhibitors, beta-blockers, and statins was strongly recommended according to the guidelines [2]. Coronary flow in the infarct-related artery before and after revascularization was graded according to the TIMI study group classification [12]. Angiographic analysis included initial and final flow of the culprit vessel. Visual assessments were performed offline in the angiographic core laboratory by 2 blinded observers (F.T., G.M).

### 2.3. CMR acquisition and analysis

CMR was performed on a 1.5-T scanner (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany) using a comprehensive protocol. All images were acquired through dedicated cardiac software, phased-array surface receiver coil and electrocardiogram triggering. All patients underwent a specific study protocol for myocardial infarction, including cine images, for functional analysis, acquired in the long and short axis by applying steady-state free precession sequences (long axis: repetition time (TR), 3.5 ms; echo time (TE), 1.2 ms; and short axis: TR, 6.0 ms; TE, 1.0 ms, slice thickness 6 mm, gap 0 mm). Subsequently, breath-hold, black-blood, T2-weighted triple inversion-recovery sequences (TR, 2 xRR; TE, 61 ms; TI 160 ms, slice thickness 7 mm) were acquired in the same slice positions as cine CMR. Postcontrast images included first-pass perfusion and late gadolinium enhancement performed in the same slice positions as cine CMR by applying gradient echo sequences (breath-held segmented protocol with 10 ms echo spacing, TE 5.0 ms and slice thickness 7 mm) 8–15 min after administration of contrast media (Gadobutrol, Gadovist; Bayer; 0.2 mmol/kg of body weight). All images were analysed using dedicated software (CVI, Circle Cardiovascular Imaging Inc, Calgary, Canada, Version 5.13.7). Left ventricle end-diastolic volume (LVEDV), end-systolic volume (LVESV), ejection fraction (LVEF) and mass were calculated from the short-axis cine images. Right ventricle end-diastolic volume (RVEDV), end-systolic volume (RVESV), and ejection fraction (RVEF) were also calculated. The presence, localization, and distribution patterns of edema and late gadolinium enhancement (LGE) were assessed at short- and long-axis images and defined as present only if detectable in both orthogonal planes. Infarct size (IS) and area at risk were identified as hyperintense regions in the delayed postcontrast and T2-weighted images, respectively, using semiautomated computer-aided threshold detection (presence in ≥10 adjacent myocardial pixels of a signal intensity >5 Ds and >2 Ds of remote myocardium for LGE and edema quantification) [13]. MVO was defined as hypoenhanced regions within the infarcted myocardium and it was considered a sign of NR. Intramyocardial hemorrhage (IMH) was defined as a central core of hypointense signal within the area of increased T2 signal intensity [14,15]. Left and right volumes and mass were normalized to body surface area. Area at risk and infarct size were expressed as a percentage of LV volume. Salvaged myocardium was quantified as the difference between the volume of increased T2-signal (area at risk) and the volume of delayed enhancement (infarct size), as previously described [15]. CMR images were blindly evaluated by two observers (M.P.M. and A.C.)

### 2.4. Laboratory assays

Blood samples were drawn from a brachial vein in all patients within 12 h following PPCI. Plasma and serum aliquots were stored at −80 °C in appropriate tubes until further analysis. ETAR-AAs were determined by enzyme-linked immunosorbent technique (ELISA) using a 96-well microtiter plate coated with extracts of ETAR-AAs in their native configuration, according to the manufacturer's instructions (CellTrend, Luckenwalde, Germany). Briefly, serum samples (diluted 1:100), standards, and positive and negative controls were added to the plate and incubated at 4 °C for 2 h. After three washing steps, plates were incubated for 60 min at room temperature with horseradish peroxidase-labelled goat anti-human IgG, followed by incubation with 100 μL of the chromogenic substrate tetramethylbenzidine (TMB) for 20 min. After blocking the reaction, the absorbance of each well was analysed at 450 nm with an ELISA reader (iEMS Reader MF Multiskan, Thermo).

Each specimen was run in duplicate. Standard curves with ETAR-AA standard points (2.5, 5, 10, 20, 40 U/mL) were included in each plate to enable the accurate quantification of antibodies in patient and control sera. Troponin I levels were measured every 4 h during the first day and every 24 h on the following 3 days using a high-sensitivity method.

### 2.5. Statistical analysis

Continuous data were presented as medians with interquartile ranges (IQRs). Comparisons between groups were performed using the Mann–Whitney *U* test for continuous variables. Categorical data were reported as the frequency with percentage, and the comparison between groups was made using the chi-square test or Fisher's exact test, as appropriate. Analyses of positive versus negative sera on an individual basis were performed according to the threshold concentrations in U/mL that were provided by the manufacturer (>10 U/mL) (CellTrend, Luckenwalde, Germany). A receiver operating characteristic (ROC) curve was generated to create a graphical representation of the diagnostic ability of ETAR-AA concentration to discriminate between STEMI patients and healthy subjects. We also decided to perform our own analysis to identify the optimal cut-off point according to the value that maximizes the sum of sensitivity and specificity in identifying MVO. The difference between groups with regard to MVO was analysed with the use of a logistic regression model. We conducted a backward stepwise approach to restrict the model to the most predictive risk factors. Risk factors were tested for retention at each step using Likelihood ratio $\chi^2$ tests. Risk factors that did not impact significantly on the model were removed. The predictive ability of the final model was quantified using the C-Index (area under the receiver operating characteristic curve, ROC) and Hosmer-Lemeshow statistic for goodness of fit. Bivariate associations between ETAR-AAs serum levels and CMR findings were described using Spearman correlation coefficients (ρ). All tests were two-sided, and the statistical significance was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 26. Figures were made in GraphPad Prism version 7.

The authors had full access to and take full responsibility for the integrity of the data. All authors read and agreed to the manuscript as written.

**Table 1**
Demographic and clinical characteristics of patients with and without ETAR-AAs.

| | Whole study population (n = 50) | ETAR-AAs negative (n = 36) | ETAR-AAs positive (n = 14) | *p* |
|---|---|---|---|---|
| Age - yr | 57 (51–69) | 59 (51–72) | 57 (54–71) | 0.611 |
| Male – no. (%) | 40 (72) | 29 (80) | 11 (78) | 0.875 |
| Smoking – no. (%) | 20 (40) | 14 (39) | 6 (43) | 0.797 |
| Diabetes mellitus – no. (%) | 6 (12) | 4 (11) | 2 (14) | 0.756 |
| Hypertension – no. (%) | 19 (38) | 12 (33) | 7 (50) | 0.276 |
| Dyslipidemia- no. (%) | 15 (30) | 12 (33) | 3 (21) | 0.409 |
| Hystory of angina – no. (%) | 4 (8) | 4 (11) | 0 (0) | 0.193 |
| Infarct location – no. (%) | | | | 0.529 |
| Anterior | 32 (64) | 24 (67) | 8 (57) | |
| Nonanterior | 18 (36) | 12 (33) | 6 (43) | |
| Peak hs-troponin I concentration – ng/L | 70,883 (40,523–197,800) | 47,639 (23,027–99,105) | 168,500 (84,243–228,350) | **0.001** |
| Pain-to-balloon time – min | 165 (118–320) | 155 (111–222) | 193 (129–330) | 0.159 |
| Initial ST elevation – mm | 4 (3–5) | 4 (2–5) | 4.5 (3.0–6.5) | 0.343 |
| ST resolution <30% – no. (%) | 26 (63) | 15 (71) | 11 (55) | 0.287 |
| Time to ST resolution – hours | 4 (2–48) | 2.5 (1.0–9.5) | 48 (2.0–96) | 0.071 |
| **Echocardiographic parameters** | | | | |
| LVEDV– mL/m$^2$ | 55 (47–64) | 54 (45–64) | 55 (51–67) | 0.601 |
| LVESV– mL/m$^2$ | 29 (22–37) | 27 (22–37) | 29 (24–34) | 0.625 |
| LVEF– % | 47 (40–55) | 49 (39–54) | 45 (39–57) | 0.842 |
| WMSI | 1.71 (1.41–2.00) | 1.71 (1.35–2.00) | 1.73 (1.41–1.95) | 0.973 |
| **Medication at admission** | | | | |
| Aspirin – no. (%) | 6 (12) | 6 (17) | 0 (0) | 0.103 |
| Beta-blocker – no. (%) | 6 (12) | 5 (14) | 1 (7) | 0.510 |
| Statins – no. (%) | 5 (10) | 5 (14) | 0 (0) | 0.142 |
| ACE inhibitor – no. (%) | 7 (14) | 5 (14) | 2 (14) | 0.971 |
| **Postprocedural results** | | | | |
| Balloon predilatation – no. (%) | 32 (63) | 25 (70) | 7 (50) | 0.198 |
| Slow or no reflow – no. (%) | 8 (16) | 3 (8) | 5 (36) | **0.017** |
| Side-branch embolization – no. (%) | 8 (16) | 4 (11) | 4 (28) | 0.130 |
| Maximal inflation pressure – atm | 12 (12–14) | 13 (12–14) | 12 (12–14) | 0.813 |
| No of stents | 1 (1–2) | 1 (1–2) | 1.5 (1–2) | 0.838 |
| TIMI flow grade 3 – no. (%) | 40 (80) | 34 (95) | 6 (43) | **0.0002** |
| **CMR characteristics** | | | | |
| Area at risk (edema), % LV | 36.8 (17.7–63.1) | 33.9 (17.8–54.4) | 50.8 (17.7–75.9) | 0.252 |
| IS, % LV | 30.1 (17.3–46.1) | 24 (17.1–45.7) | 35.8 (22.3–48.5) | 0.210 |
| Myocardial salvage, % LV | 6.6 (0.9–19.7) | 5.6 (0–15.9) | 10.1 (2.3–17.9) | 0.273 |
| MVO – no. (%) | 24 (48) | 14 (39) | 10 (71) | **0.030** |
| IMH – no. (%) | 20 (40) | 12 (33) | 9 (64) | **0.048** |
| LVEDV, mL/m$^2$ | 88 (78–99) | 86 (78–111) | 95 (79–99) | 0.651 |
| LVESV, mL/m$^2$ | 45 (37–58) | 44 (34–61) | 46 (43–58) | 0.315 |
| LVEF, % | 48 (38–55) | 51 (38–56) | 44 (41–51) | 0.302 |
| LVMI, g/m$^2$ | 57 (49–64) | 57 (49–65) | 59 (49–64) | 0.763 |
| RVEDV, mL/m$^2$ | 72 (64–80) | 72 (64–81) | 72 (52–79) | 0.970 |
| RVESV, mL/m$^2$ | 31 (24–36) | 29 (24–36) | 33 (24–45) | 0.268 |
| RVEF, % | 56 (54–61) | 58 (55–64) | 54 (53–56) | **0.006** |

ACE = angiotensin-converting enzyme; CMR = cardiac magnetic resonance; IMH = intramyocardial hemorrhage; IS = infarct size; LV = left ventricle; LVEDV = left ventricle end-diastolic volume; LVEF = left ventricle ejection fraction; LVESV = left ventricle end-systolic volume; MVO = microvascular obstruction; WMSI = wall motion score index.

## 3. Results

A total of 50 patients with STEMI were recruited and underwent peripheral venous blood sampling after PPCI to evaluate the levels of ETAR-AAs. Table 1 lists the baseline clinical characteristics of these patients. No patient had autoimmune diseases. The median pre-PPCI time was 165 min (range 118–320). Most patients (80%) were men, with a mean age of 59 ± 11 years (range 32–85), and experienced predominantly left anterior descending artery infarcts (64%). The peak hs-troponin I concentration was 70,883 ng/L (40,523–197,800). A final TIMI flow 3 was achieved in 42 patients (84%). Angiographic slow or no-reflow was observed in 8 patients (16%). Side-branch embolization was observed in 8 patients (16%).

### 3.1. Antibody concentrations in STEMI patients vs. healthy subjects

There were no significant differences between STEMI patients and healthy subjects in median autoantibody concentration (7.1 U/mL [4.5–10.7] *vs.* 7.6 U/mL [5.9–10.3] $p = 0.383$). The autoantibody test had no significant ability to discriminate between STEMI patients and healthy subjects. ETAR-AAs provided a C statistic of 0.554 (95% CI 0.435–0.673, $p = 0.381$). There were no significant differences in the proportion of participants in each group (STEMI patients and healthy subjects) who were seropositive on the basis of the manufacturer-provided antibody concentration threshold ($p = 0.476$) (Supplementary Fig. 1).

### 3.2. Characteristics of patients with ETAR-AAs seropositivity (>10 U/mL)

Table 1 summarizes the results observed with ETAR-AAs seropositive (n = 14, 28%) and ETAR-AAs seronegative (n = 36, 72%) patients. We have not recorded any cases of in-hospital death or cardiogenic shock. Two patients with ETAR-AAs seropositivity arrived at the hospital with pulmonary edema and two patients had ventricular fibrillation before reperfusion. Amongst the latter patients, in one case ETAR-AAs levels were high (23.1 U/mL), in the other case ETAR-AAs levels were low (4.5 U/mL).

### 3.3. CMR findings in patients with ETAR-AAs seropositivity (>10 U/mL)

All CMR characteristics in patients with and without ETAR-AAs are listed in Table 1. In multivariable logistic regression analysis, ETAR-AAs seropositivity was a significant predictor of MVO ($p = 0.03$) together with IS ($p = 0.01$) (Table 2). The multivariable model significantly predicted the occurrence of MVO (model $\chi^2 = 21.060$, probability value $< 0.001$). The model discriminated well between patients who did and did not develop MVO (ROC: 0.843; 95% CI: 0.730 to 0.7956, $p < 0.0001$) (Supplementary Fig. 2A). The model was well calibrated between observed and expected risk (Hosmer-Lemeshow $\chi^2 = 10.879$, probability value 0.209). Observed and expected probability was highly correlated ($R = 0.893$, probability value $< 0.001$) (Supplementary Fig. 2B).

### 3.4. Categorical seropositivity using the ROC-derived ETAR-AA threshold

ETAR-AAs were higher in patients with MVO (8.9 U/mL [6.8–16. 2] *vs.* 5.7 U/mL [4.3–7.7], $p = 0.002$) (Fig. 1A). A value of ≥6.74 U/mL was identified as the optimal cut-off point for MVO prediction, providing an area under the ROC curve of 0.742 (SE 0.071; 95% confidence interval 0.601–0.882; $p = 0.003$) (sensitivity 79%; specificity 65%; NPV 71%; PPV 74%; accuracy 72%) (Fig. 1B). The prevalence of MVO was 79% in patients with ETAR-AAs ≥6.74 U/mL and 22% in patients with ETAR-AAs <6.74 U/mL ($p = 0.002$). The prevalence of IMH was higher in patients with ETAR-AAs ≥6.74 U/mL (76% *vs.* 41%, $p = 0.01$). Wall motion score index was higher in patients with ETAR-AAs ≥6.74 U/mL ($p = 0.04$). On CMR, infarct size was larger in patients with ETAR-AAs ≥6.74 U/mL ($p = 0.02$). LVESV and RVESV were greater in patients with ETAR-AAs ≥6.74 U/mL ($p = 0.009$ and $p = 0.027$, respectively). LVEF and RVEF were lower in patients with ETAR-AAs ≥6.74 U/mL ($p = 0.009$ and $p = 0.007$, respectively).

### 3.5. Bivariate correlation of ETAR-AAs serum concentration with CMR findings

ETAR-AAs serum titers were directly correlated with MVO and IMH extension ($\rho = 0.464$, $p < 0.001$ and $\rho = 0.302$, $p = 0.02$, respectively). ETAR-AAs serum titers were inversely correlated witho LVEF and RVEF ($\rho = -0.350$, $p = 0.01$ and $\rho = -0.489$, $p < 0.001$, respectively). Moreover, ETAR-AAs serum titers were directly correlated with peak hs-

**Table 2**
Univariable and multivariable predictors of MVO.

| Covariates | Univariable analysis | | | Multivariable analysis[a] | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* | OR | 95% CI | *p* |
| ETAR-AAs >10 U/mL | 3.91 | 1.02–14.91 | **0.020** | 3.22 | 1.31–7.11 | **0.032** |
| Age | 0.97 | 0.93–1.02 | 0.342 | | | |
| Sex | 0.90 | 0.22–3.61 | 0.887 | | | |
| Hypertension | 0.52 | 0.16–1.67 | 0.275 | | | |
| Diabetes | 0.15 | 0.01–1.41 | 0.098 | | | |
| Smoke | 0.87 | 0.28–2.71 | 0.817 | | | |
| Dyslipidemia | 3.66 | 0.97–13.8 | 0.071 | | | |
| Chronic kidney disease | 0.92 | 0.90–1.12 | 0.777 | | | |
| IS | 1.09 | 1.04–1.15 | **0.001** | 1.06 | 1.01–1.12 | **0.019** |
| Final TIMI flow grade <2 | 6.43 | 1.41–28.55 | **0.015** | 2.11 | 0.17–25.61 | 0.558 |
| Thrombus burden | 1.92 | 0.98–3.76 | 0.065 | | | |
| Side-branch embolization | 1.18 | 0.23–6.11 | 0.839 | | | |
| Anterior STEMI | 3.84 | 1.08–13.37 | **0.036** | 6.42 | 1.5–66.50 | 0.113 |
| Glycoprotein IIb/IIIa inhibitor | 9.62 | 1.05–11.75 | **0.046** | 0.54 | 0.08–3.92 | 0.577 |
| Pain-to-ballon time | 1.00 | 0.99–1.00 | 0.474 | | | |
| Pre-infarction angina | 3.00 | 0.29–31.0 | 0.357 | | | |
| BNP on admission | 0.99 | 0.99–1.00 | 0.313 | | | |

[a]Multivariable logistic regression analysis was applied to identify whether ETAR-AAs were independently associated with MVO. At this scope, in the model we included variables showing a significant association with MVO at univariable analysis (*p* values < 0.05). The assumption of linearity for continuous variables included in the model was confirmed by logit step test. Hosmer and Lemeshow test: Chi-square 10.879, $p = 0.209$. $R^2$ of the model 0.797.
BNP, brain natriuretic peptide; IS, infarct size; MVO, microvascular obstruction; STEMI, ST-elevation myocardial infarction. Other abbreviations as in Table 1.

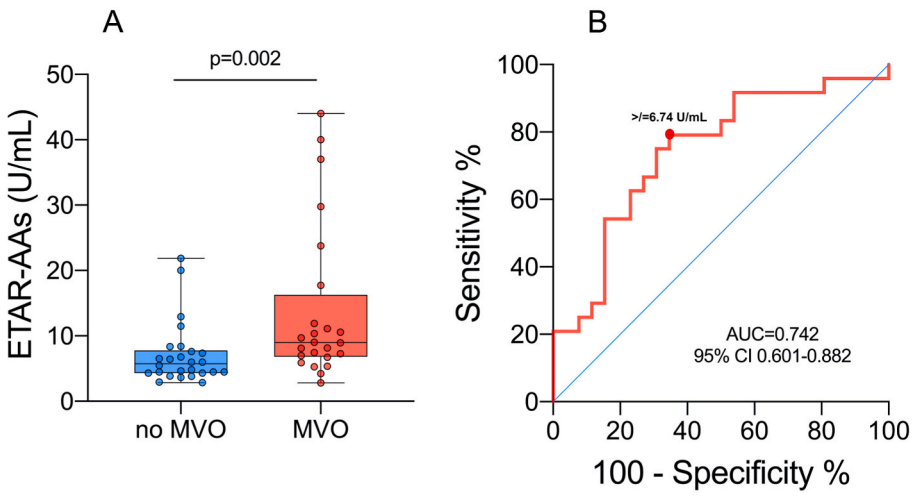


**Fig. 1.** ETAR-AAs concentration in STEMI patients with MVO and ROC curve for ETAR-AAs in STEMI patients with MVO versus patients without MVO.
(A) ETAR-AAs concentration (U/mL) in STEMI patients with MVO and without MVO. Values are expressed as median and IQR. (B) Data are presented as area under the curve (AUC) and 95% CI. A greater AUC indicates greater ability for the concentration of ETAR-AAs to discriminate whether a STEMI patient will have MVO. If 0.5 is contained within the 95% CI, then there is no significant difference between STEMI patients with MVO and patients without. ETAR, autoantibodies activating endothelin-1 receptors type A; MVO, microvascular obstruction; STEMI, ST-elevation myocardial infarction.

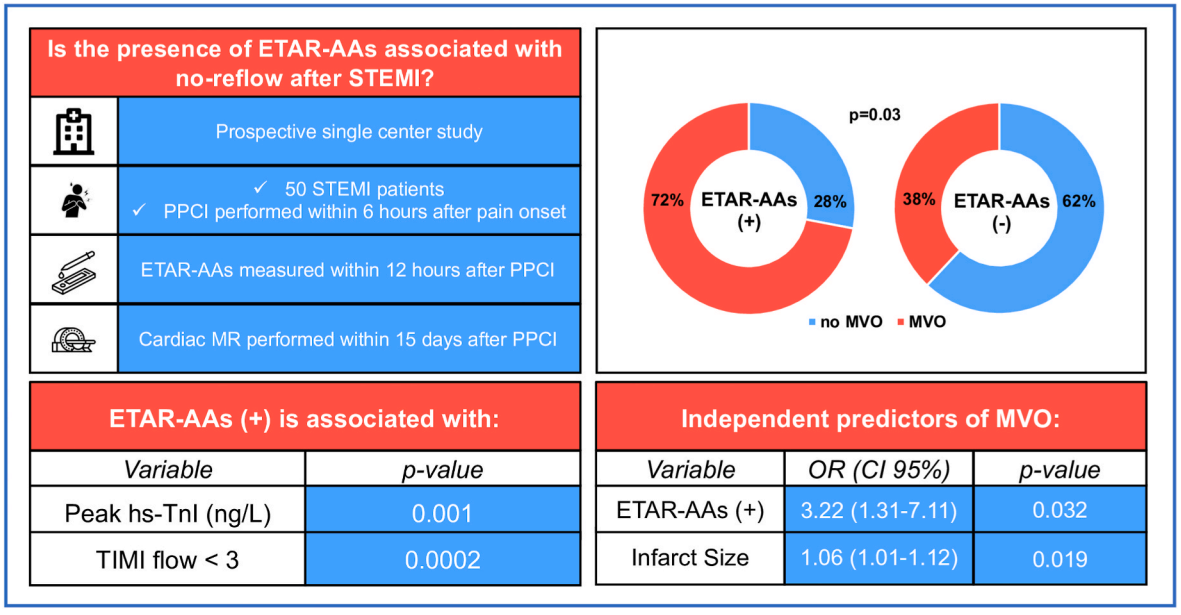


**Fig. 2.** No-reflow, where the coronary artery is patent after treatment of STEMI but tissue perfusion is not restored, is associated with worse outcomes.
This is a prospective study conducted at Padua University Hospital between January 2022 and June 2022. We aimed to investigate the relationship between ETAR-AAs and no-reflow after PPCI in STEMI. No-reflow was assessed by cardiac magnetic resonance imaging (MVO, microvascular obstruction). The prevalence of MVO was higher in patients with ETAR-AAs seropositivity (72% *vs.* 38%, $p = 0.03$). ETAR-AAs seropositivity was independently associated with no-reflow ($p = 0.03$). The ETAR-AA-triggered pathway is a risk factor for no-reflow in STEMI patients. ETAR-AAs, autoantibodies targeting endothelin-1 type A receptor; hs TnI, high sensitivity troponin I; MR, magnetic resonance; MVO, microvascular obstruction; PPCI, primary percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction.

troponin I concentration ($\rho = 0.361$, $p = 0.01$), and LVESV ($\rho = 0.361$, $p = 0.01$).

## 4. Discussion

In this study, we demonstrate, for the first time, that in STEMI patients, ETAR-AA serum titers are associated with MVO after successful PPCI. This association appears to be independent of potential confounding variables (Fig. 2).

The NR phenomenon refers to severe MVO that is known to be associated with impaired LV function and poor prognosis in patients undergoing successful PPCI [4]. Although it occurs in approximately 40% of STEMI patients undergoing PPCI at varying intensity, identification of coronary microvascular injury depends on the diagnostic capability of the method used in its detection. CMR is the gold standard technique that allows for the accurate visualization of regions with MVO within the infarcted area [15]. In the present study, the prevalence of MVO was 48%, which is consistent with previous reports using CMR [16].

The exact mechanism underlying NR in humans is still poorly understood. The most accredited hypotheses are related to endothelial dysfunction and alterations in the microvascular circulation [3,17–19]. Specifically, NR is related to a functional and structural alteration of the coronary microcirculation and the main pathophysiological mechanisms include distal atherothrombotic embolization, ischemic damage and reperfusion injury [3,17]. Moreover, the presence of preexisting endothelial dysfunction increases the susceptibility to microvascular dysfunction and NR [20].

ETAR-AAs have been demonstrated to have a detrimental effect on the endothelium [9]. They stimulate vasoconstriction, induce activation of human microvascular endothelial cells, increase secretion of proinflammatory chemokines and cause damage by promoting migration of immune cells to target tissues [9]. Therefore, we hypothesized that ETAR-AAs may be involved in NR.

In our study, the finding that no significant differences in ETAR-AAs serum concentrations, categorical seropositivity rates, and ROC curve between patients with STEMI and healthy subjects, together with the short time (hours) elapsed between reperfusion and serum withdrawal for ETAR-AA measurements, suggests the preexistence of such autoantibodies in patients with STEMI and that these did not arise after reperfusion. This is not surprising. Indeed, in other clinical settings, the preexistence of ETAR-AAs predicts the future occurrence of cardiovascular manifestations [10,11]. Under a strictly immunological point of view, we must take for granted the preexistence of the ETAR-AAs in STEMI patients. Indeed, because it takes a while for the human immune system to generate antibodies against a new antigen, it is only possible to detect antibodies in the blood from about two weeks after antigen exposure onward [21]. In line with our results, the finding that ETAR-AA concentrations are not different between patients and healthy subjects has been recently reported [22]. This finding is not unexpected as autoantibodies are hallmark findings in many autoimmune diseases that are often detected prior to disease onset [23]. Moreover, autoantibodies may exist in healthy subjects at the same concentrations that are observed in patients with autoimmune disease [24].

How are ETAR-AAs activated? To serve as antigens, GPCRs must be degraded to small oligopeptides, and one or more degradation products must be able to form a complex with one of the HLA class II molecules. Antigenic determinants from targets, which are protected against immune attack under physiologic conditions, may become accessible after injury to the target tissue. Subsequent liberation and presentation of target antigens to the immune system may then induce an autoimmune response that is precipitated in various conditions [25].

In our study, ETAR-AAs were independent predictors of MVO. Why would ETAR-AAs play such a detrimental role in the acute setting, having been silent previously for the entire life of the patient? At this stage, we do not have a precise answer to this question, and answering this question goes beyond the scope of our present study. However, we cannot exclude that the presence of ETAR-AAs might be associated with preexisting subclinical coronary microvascular dysfunction, which can make coronary microvasculature more susceptible to NR. On the other hand, it is possible that the expression of ETARs in the infarct zone may change. Indeed, in murine models, ETAR expression increases in infarcted areas after myocardial infarction [26]. We can hypothesize that the already well-described surge in endothelin levels [19,27] can stimulate ETAR expression, similar to what happens with angiotensin II and AT1R [28]. Variations in autoantibody function may likewise contribute to explaining our results. Specific metabolic conditions, such as those induced by hypoxia, ischaemia, and/or inflammation, could be prerequisites to the realization of the full activity of ETAR-AAs [29]. Indeed, we cannot rule out at this stage that quiescent autoantibodies may be activated as a consequence of myocardial infarction. Therefore, we hypothesize that during ischaemia/reperfusion, ETAR-AAs may act as a trigger for the NR phenomenon. In line with this hypothesis, some authors have recently observed that ETAR-AAs are natural components of the immune system and may become dysregulated, triggering autoimmune processes [24]. This assumption is in accordance with the emerging evidence of the role of the immune system in homeostasis beyond host defence [30]. Specifically, it has been demonstrated that ETAR-AAs stimulate neutrophil migration [31]. Neutrophils are the most abundant peripheral blood-circulating leukocytes and the first white cells to invade sites of tissue damage and inflammation, even in NR [5,32].

Supporting our hypothesis, in the clinical setting of solid organ transplantation, ETAR-AAs have been demonstrated to be involved in vascular rejection [33–35]. Vascular antibody-mediated rejection is characterized by capillaritis with diffuse blood extravasation, thrombosis inside small arteries and lymphomonocyte infiltration around arterioles. These histological features have been demonstrated in transplant recipients with ETAR-AA positivity that is detectable even before transplantation [34,35] and are also pathological features described in the NR phenomenon [36–38]. Contributing to these common pathological features is the fact that NR and transplantation share ischaemia–reperfusion injury [29]. In the context of transplantation, ETAR-AAs bind to the extracellular portion of the ETARs that is exposed following endothelium damage [39].

### 4.1. Limitations

This study has some limitations. First, the sample size is small, and therefore, our data need confirmation in future studies involving a larger patient cohort. Nevertheless, the statistical significance related to the association between ETAR-AAs and MVO was obtained even after adjustment for the other clinical and angiographic variables, thus strongly suggesting that ETAR-AAs may actually have a role in the onset of NR. Second, our study is a hypothesis-generating study, and firm conclusions about the causal relationship between ETAR-AAs and MVO cannot be drawn. To confirm our hypothesis further preclinical modelling is needed. The final proof of a pathogenetic role of ETAR-AAs in the NR phenomenon will require the passive transfer of ETAR-AA-positive sera from patients to animal models of ischaemia–reperfusion, according to Rose–Witebsky autoimmunity criteria.

### 4.2. Conclusions

Our results support the hypothesis that ETAR-AAs seropositivity is associated with NR in STEMI patients. These initial observations may set the stage for a better pathophysiological understanding of the mechanisms contributing to NR with important implications in research and clinical care. Future studies are needed to further characterize the role of anti-ETAR autoimmunity in NR.

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## CRediT authorship contribution statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2023.06.970.

## References

[1] C.W. Tsao, A.W. Aday, Z.I. Almarzooq, A. Alonso, A.Z. Beaton, et al., On behalf of the American heart association council on epidemiology and prevention statistics committee and stroke statistics subcommittee. Heart disease and stroke statistics—2022 update: a report from the American heart association, Circulation (2022) 145.
[2] P.T. O'Gara, F.G. Kushner, D.D. Ascheim, D.E. Casey, M.K. Chung, et al., ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American college of cardiology foundation/American heart association task force on practice guidelines, Circulation 2013 (127) (2013).
[3] B.G. Schwartz, R.A. Kloner, Coronary no reflow, J. Mol. Cell. Cardiol. 52 (2012) 873–882.
[4] A.M. Maznyczka, P.J. McCartney, K.G. Oldroyd, M. Lindsay, M. McEntegart, et al., Effects of intracoronary alteplase on microvascular function in acute myocardial infarction, J. Am. Heart Assoc. 9 (2020), e014066.
[5] J.E. Jordan, Z.Q. Zhao, J. Vinten-Johansen, The role of neutrophils in myocardial ischemia-reperfusion injury, Cardiovasc. Res. 43 (1999) 860–878.
[6] J. Li, H. Zhang, C. Zhang, Role of inflammation in the regulation of coronary blood flow in ischemia and reperfusion: mechanisms and therapeutic implications, J. Mol. Cell. Cardiol. 52 (2012) 865–872.
[7] T. Horinouchi, K. Terada, T. Higashi, S. Miwa, Endothelin receptor signaling: new insight into its regulatory mechanisms, J. Pharmacol. Sci. 123 (2013) 85–101.
[8] G. Civieri, L. Iop, F. Tona, Antibodies against angiotensin II type 1 and endothelin 1 type A receptors in cardiovascular pathologies, Int. J. Mol. Sci. 23 (2022) 927.
[9] M.C. Philogene, T. Johnson, A.J. Vaught, S. Zakaria, N. Fedarko, Antibodies against angiotensin II type 1 and endothelin A receptors: relevance and pathogenicity, Hum. Immunol. 80 (2019) 561–567.
[10] G. Riemekasten, A. Philippe, M. Näther, T. Slowinski, D.N. Müller, et al., Involvement of functional autoantibodies against vascular receptors in systemic sclerosis, Ann. Rheum. Dis. 70 (2011) 530–536.
[11] O. Cabral-Marques, G. Riemekasten, Vascular hypothesis revisited: role of stimulating antibodies against angiotensin and endothelin receptors in the pathogenesis of systemic sclerosis, Autoimmun. Rev. 15 (2016) 690–694.
[12] TIMI Study Group, The thrombolysis in myocardial infarction (TIMI) trial. Phase I findings, N. Engl. J. Med. 312 (1985) 932–936.
[13] I. Eitel, S. Desch, G. Fuernau, L. Hildebrand, M. Gutberlet, et al., Prognostic significance and determinants of myocardial salvage assessed by cardiovascular magnetic resonance in acute reperfused myocardial infarction, J. Am. Coll. Cardiol. 55 (2010) 2470–2479.
[14] K.C. Wu, R.J. Kim, D.A. Bluemke, C.E. Rochitte, E.A. Zerhouni, et al., Quantification and time course of microvascular obstruction by contrast-enhanced echocardiography and magnetic resonance imaging following acute myocardial infarction and reperfusion, J. Am. Coll. Cardiol. 32 (1998) 1756–1764.
[15] A. Demirkiran, H. Everaars, R.P. Amier, C. Beijnink, M.J. Bom, et al., Cardiovascular magnetic resonance techniques for tissue characterization after acute myocardial injury, Eur Heart J Cardiovasc Imaging 20 (2019) 723–734.
[16] R. Nijveldt, A.M. Beek, A. Hirsch, M.G. Stoel, M.B.M. Hofman, et al., Functional recovery after acute myocardial infarction, J. Am. Coll. Cardiol. 52 (2008) 181–189.
[17] L.S.F. Konijnenberg, P. Damman, D.J. Duncker, R.A. Kloner, R. Nijveldt, et al., Pathophysiology and diagnosis of coronary microvascular dysfunction in ST-elevation myocardial infarction, Cardiovasc. Res. 116 (2020) 787–805.
[18] D.J. Hausenloy, W. Chilian, F. Crea, S.M. Davidson, P. Ferdinandy, et al., The coronary circulation in acute myocardial ischaemia/reperfusion injury: a target for cardioprotection, Cardiovasc. Res. 115 (2019) 1143–1155.
[19] L. Galiuto, A.N. DeMaria, U del Balzo, K. May-Newman, S.F. Flaim, et al., Ischemia-reperfusion injury at the microvascular level: treatment by endothelin A–selective antagonist and evaluation by myocardial contrast echocardiography, Circulation 102 (2000) 3111–3116.
[20] R.A. Kloner, K.S. King, M.G. Harrington, No-reflow phenomenon in the heart and brain, Am. J. Physiol. Heart Circ. Physiol. 315 (2018) H550–H562.
[21] J. Parkin, B. Cohen, An overview of the immune system, Lancet 357 (2001) 1777–1789.
[22] J. Hall, K.M. Bourne, S. Vernino, V. Hamrefors, I. Kharraziha, et al., Detection of G Protein–Coupled receptor autoantibodies in postural orthostatic tachycardia syndrome using standard methodology, Circulation 146 (2022) 613–622.
[23] Z.X. Xiao, J.S. Miller, S.G. Zheng, An updated advance of autoantibodies in autoimmune diseases, Autoimmun. Rev. 20 (2021), 102743.
[24] O. Cabral-Marques, A. Marques, L.M. Giil, R. De Vito, J. Rademacher, et al., GPCR-specific autoantibody signatures are associated with physiological and pathological immune homeostasis, Nat. Commun. 9 (2018) 5224.
[25] D. Dragun, A. Philippe, R. Catar, B. Hegner, Autoimmune mediated G-protein receptor activation in cardiovascular and renal pathologies, Thromb. Haemostasis 101 (2009) 643–648.
[26] M.A. Kimm, H. Haas, M. Stölting, M. Kuhlmann, C. Geyer, et al., Targeting endothelin receptors in a murine model of myocardial infarction using a small molecular fluorescent probe, Mol. Pharm. 17 (2020) 109–117.
[27] G. Niccoli, Endothelin-1 and acute myocardial infarction: a no-reflow mediator after successful percutaneous myocardial revascularization, Eur. Heart J. 27 (2006) 1793–1798.
[28] R. Eckenstaler, J. Sandori, M. Gekle, R.A. Benndorf, Angiotensin II receptor type 1 – an update on structure, expression and pathology, Biochem. Pharmacol. 192 (2021), 114673.
[29] M.J.M. Silvis, S.E. Kaffka genaamd Dengler, C.A. Odille, M. Mishra, et al., Damage-associated molecular patterns in myocardial infarction and heart transplantation: the road to translational success, Front. Immunol. 11 (2020), 599511.
[30] P. Matzinger, The danger model: a renewed sense of self, Science 296 (2002) 301–305.
[31] S de Oliveira, E.E. Rosowski, A. Huttenlocher, Neutrophil migration in infection and wound repair: going forward in reverse, Nat. Rev. Immunol. 16 (2016) 378–391.
[32] C. Duilio, G. Ambrosio, P. Kuppusamy, A. DiPaula, L.C. Becker, et al., Neutrophils are primary source of O2 radicals during reperfusion after prolonged myocardial ischemia, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H2649–H2657.
[33] B.J. Nankivell, M. Shingde, C.H. P'Ng, A. Sharma, The clinical and pathologic phenotype of antibody-mediated vascular rejection diagnosed using arterial C4d immunoperoxidase, Kidney Int Rep 7 (2022) 1653–1664.
[34] N.E. Hiemann, R. Meyer, E. Wellnhofer, C. Schoenemann, H. Heidecke, et al., Non-HLA antibodies targeting vascular receptors enhance alloimmune response and microvasculopathy after heart transplantation, Transplantation 94 (2012) 919–924.
[35] E. Cozzi, F. Calabrese, M. Schiavon, P. Feltracco, M. Seveso, et al., Immediate and catastrophic antibody-mediated rejection in a lung transplant recipient with anti-angiotensin II receptor type 1 and anti-endothelin-1 receptor type A antibodies, Am. J. Transplant. 17 (2017) 557–564.
[36] R.A. Kloner, R.E. Rude, N. Carlson, P.R. Maroko, L.W. DeBoer, et al., Ultrastructural evidence of microvascular damage and myocardial cell injury after coronary artery occlusion: which comes first? Circulation 62 (1980) 945–952.
[37] A. Krug, Mesnil Du, de Rochemont null, G. Korb, Blood supply of the myocardium after temporary coronary occlusion, Circ. Res. 19 (1966) 57–62.
[38] R.A. Kloner, C.E. Ganote, R.B. Jennings, The "No-reflow" phenomenon after temporary coronary occlusion in the dog, J. Clin. Invest. 54 (1974) 1496–1508.
[39] Q. Zhang, E.F. Reed, The importance of non-HLA antibodies in transplantation, Nat. Rev. Nephrol. 12 (2016) 484–495.