

## Original article

## EpCAM and microvascular obstruction in patients with STEMI: a cardiac magnetic resonance study



César Ríos-Navarro,<sup>a</sup> José Gavara,<sup>a</sup> Julio Núñez,<sup>a,b,c,d</sup> Elena Revuelta-López,<sup>e</sup> José V. Monmeneu,<sup>f</sup> María P. López-Lereu,<sup>f</sup> Elena de Dios,<sup>c,d</sup> Nerea Pérez-Solé,<sup>a</sup> José M. Vila,<sup>a,g</sup> Ricardo Oltra,<sup>h</sup> Francisco J. Chorro,<sup>a,b,c,d</sup> Antoni Bayés-Genís,<sup>c,e</sup> and Vicente Bodi<sup>a,b,c,d,\*</sup>

<sup>a</sup>Instituto de Investigación Sanitaria INCLIVA, Valencia, Spain

<sup>b</sup>Departamento de Cardiología, Hospital Clínico Universitario de Valencia, Valencia, Spain

<sup>c</sup>Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Spain

<sup>d</sup>Departamento de Medicina, Facultad de Medicina, Universidad de Valencia, Valencia, Spain

<sup>e</sup>Instituto del Corazón, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

<sup>f</sup>Unidad de Resonancia Magnética Cardiovascular, Exploraciones Radiológicas Especiales (ERESA), Valencia, Spain

<sup>g</sup>Departamento de Fisiología, Facultad de Medicina, Universidad de Valencia, Valencia, Spain

<sup>h</sup>Unidad de Cuidados Intensivos, Hospital Clínico Universitario de Valencia, Valencia, Spain

## Article history:

Received 4 December 2020

Accepted 15 April 2021

Available online 25 May 2021

## Keywords:

Epithelial cell adhesion molecule

Microvascular obstruction

Cardiac magnetic resonance

ST-segment elevation myocardial infarction

## ABSTRACT

**Introduction and objectives:** Microvascular obstruction (MVO) is negatively associated with cardiac structure and worse prognosis after ST-segment elevation myocardial infarction (STEMI). Epithelial cell adhesion molecule (EpCAM), involved in epithelium adhesion, is an understudied area in the MVO setting. We aimed to determine whether EpCAM is associated with the appearance of cardiac magnetic resonance (CMR)-derived MVO and long-term systolic function in reperfused STEMI.

**Methods:** We prospectively included 106 patients with a first STEMI treated with percutaneous coronary intervention, quantifying serum levels of EpCAM 24 hours postreperfusion. All patients underwent CMR imaging 1 week and 6 months post-STEMI. The independent correlation of EpCAM with MVO, systolic volume indices, and left ventricular ejection fraction was evaluated.

**Results:** The mean age of the sample was  $59 \pm 13$  years and 76% were male. Patients were dichotomized according to median EpCAM (4.48 pg/mL). At 1-week CMR, lower EpCAM was related to extensive MVO ( $P = .021$ ) and larger infarct size ( $P = .019$ ). At presentation, EpCAM values were significantly associated with the presence of MVO in univariate (OR, 0.58; 95%CI, 0.38–0.88;  $P = .011$ ) and multivariate logistic regression models (OR, 0.55; 95%CI, 0.35–0.87;  $P = .010$ ). Although MVO tends to resolve at chronic phases, decreased EpCAM was associated with worse systolic function: reduced left ventricular ejection fraction ( $P = .009$ ) and higher left ventricular end-systolic volume ( $P = .043$ ).

**Conclusions:** EpCAM is associated with the occurrence of CMR-derived MVO at acute phases and long-term adverse ventricular remodeling post-STEMI.

© 2021 Published by Elsevier España, S.L.U. on behalf of Sociedad Española de Cardiología.

## EpCAM y obstrucción microvascular en pacientes con un IAMCEST: estudio con resonancia magnética cardíaca

## RESUMEN

**Introducción y objetivos:** La obstrucción microvascular (OMV) se asocia negativamente con la estructura cardíaca y el pronóstico de los pacientes tras un infarto agudo de miocardio con elevación del segmento ST (IAMCEST). El factor *epithelial cell adhesion molecule* (EpCAM), implicado en la cohesión de las células epiteliales, está poco estudiado en el contexto de la OMV. Por ello, el objetivo de este estudio es evaluar en una cohorte de pacientes con IAMCEST la asociación entre la concentración de EpCAM circulante con la extensión de la OMV, determinada por resonancia magnética cardíaca (RMC), y la función sistólica en fases crónicas.

**Métodos:** Se incluyó prospectivamente a 106 pacientes con un primer IAMCEST tratados con angioplastia primaria percutánea. La concentración sérica de EpCAM se determinó 24 h tras la reperfusión coronaria. Se estudió a todos los pacientes mediante RMC a la semana y a los 6 meses del IAMCEST. Se evaluó la correlación entre los valores de EpCAM circulante con la OMV, los índices de función sistólica y la fracción de eyección del ventrículo izquierdo.

## Palabras clave:

Epithelial cell adhesion molecule

Obstrucción microvascular

Resonancia magnética cardíaca

Infarto agudo de miocardio con elevación del segmento ST

\* Corresponding author: Departamento de Cardiología, Hospital Clínico Universitario, Avda. Blasco Ibáñez 17, 46010 Valencia, Spain.  
E-mail address: vicente.bodi@uv.es (V. Bodi).

**Resultados:** La media de edad de la cohorte era  $59 \pm 13$  años y el 76% eran varones. Se dicotomizó a los pacientes según la mediana de EpCAM (4,48 pg/ml). Se observó que los pacientes que tenían valores más bajos de EpCAM presentaban una mayor extensión de la OMV ( $p = 0,021$ ) y un mayor tamaño de infarto ( $p = 0,019$ ) en los estudios de RMC realizados 1 semana después del evento cardiovascular. Respecto a las variables de presentación, la concentración de EpCAM se asoció significativamente con la presencia de OMV en análisis de regresión logística binaria univariable (OR = 0,58; IC95%, 0,38-0,88;  $p = 0,011$ ) y multivariable (OR = 0,55; IC95%, 0,35-0,87;  $p = 0,010$ ). A pesar de que la OMV tiende a resolverse espontáneamente en fases crónicas, unos valores más bajos de EpCAM se correlacionaron con una peor función sistólica: fracción de eyección del ventrículo izquierdo deprimida ( $p = 0,009$ ) y mayor volumen telesistólico del ventrículo izquierdo ( $p = 0,043$ ).

**Conclusiones:** Tras un IAMCEST, la concentración de EpCAM circulante en fase aguda se asocia con la extensión de la OMV evaluada mediante RMC y con un peor remodelado ventricular en fase crónica.

© 2021 Publicado por Elsevier España, S.L.U. en nombre de Sociedad Española de Cardiología.

### Abbreviations

CMR: cardiac magnetic resonance  
 EpCAM: epithelial cell adhesion molecule  
 LV: left ventricle  
 LVEF: left ventricular ejection fraction  
 MVO: microvascular obstruction  
 STEMI: ST-segment elevation myocardial infarction

## INTRODUCTION

Coronary reperfusion, mainly through percutaneous techniques, markedly diminishes acute mortality after reperfused ST-segment elevation myocardial infarction (STEMI). Despite complete reperfusion at the epicardial level, however, a considerable number of patients (30% to 50%) display abnormal myocardial perfusion.<sup>1,2</sup> This phenomenon is known as microvascular obstruction (MVO) and is associated with compromised structure and function as well as with worse prognosis.<sup>3,4</sup>

Certain baseline characteristics are useful in predicting the extent of MVO upon patient arrival. Indeed, a scoring method including 4 predisposing factors (reperfusion delay, diabetes mellitus, Killip class, and sum of ST-segment elevation) at the initial electrocardiogram has been developed to predict MVO appearance.<sup>5</sup> However, soluble biomarkers to predict the extent of MVO are scarce, highlighting a crucial need for novel molecules to gain greater insight into MVO pathophysiology and provide new therapeutic alternatives.

Although several underlying mechanisms act both simultaneously and sequentially in MVO pathophysiology, endothelial abnormalities, decreased capillary density and increased endothelial permeability are particularly important.<sup>2,6,7</sup> In this context, epithelial cell adhesion molecule (EpCAM) is found in the basolateral membrane of numerous normal epithelial cells to maintain cell-to-cell adhesion, thus preserving epithelial integrity.<sup>8</sup> Despite its promising role in oncology,<sup>9</sup> the involvement of this molecule in the cardiovascular scenario is understudied.

The aim of this work was to explore the association of circulating EpCAM determined 24 hours after percutaneous coronary intervention with cardiac magnetic resonance (CMR)-derived MVO, as well as with cardiac structure and function within the first 6 months after STEMI.

## METHODS

### Study population

This study conformed to the principles for use of human subjects outlined in the Declaration of Helsinki, the study protocol

was approved by the local Research Ethics Committee, and written informed consent was obtained from all participants.

We prospectively enrolled patients discharged between July 2013 and December 2017 with the following inclusion criteria: patients with a first STEMI as outlined in current definitions<sup>10</sup> treated with percutaneous coronary intervention within 12 hours of chest pain onset, and who underwent CMR imaging 1 week and 6 months post-STEMI. Exclusion criteria were death, reinfarction, or clinical instability during the first 6 months postdischarge or any contraindication to CMR.

### Biomarker assays

Blood samples were isolated 24 hours after coronary revascularization, centrifuged at 2300 rpm for 15 minutes, and serum was immediately stored at  $-80^{\circ}\text{C}$  until further analyses were performed. Serum EpCAM levels were analyzed using the Olink CARDIOVASCULAR III panel (Olink Proteomics, Uppsala, Sweden). The Proximity Extension Assay technology used for the Proseek Multiplex protocol has been described elsewhere<sup>11</sup> or in the [supplementary data](#).

High-sensitivity troponin T (Elecsys TnT assay, Roche Diagnostics, Basel, Switzerland; myocardial infarction diagnosis cutoff  $> 14$  ng/mL) was measured on admission, at 3 hours, and at 6- to 8-hour intervals for the first 24 hours.

### White blood cell counts

Lymphocyte, and eosinophil ( $\times 1000$  cells/mL) counts were measured upon patient arrival, and at 12, 24, 48, 72, and 96 hours after revascularization using a commercial assay based on cytochemical light scatter and light absorption measurements (ADVIA 120 Hematology System from Siemens Diagnostics, Germany).

### Cardiac magnetic resonance studies

Two CMR studies (1.5 T unit, Magnetom Sonata; Siemens, Erlangen, Germany) were performed at 7 [5–8] days (1-week CMR) and 196 [167–242] days (6-month CMR) after STEMI, in accordance with our laboratory protocol<sup>12</sup> and current recommendations. All studies were performed and analyzed by 2 cardiologists specialized in CMR with 15 years of experience, using customized software (QMASS MR 6.1.5, Medis, Leiden, The Netherlands). CMR data were prospectively recorded and immediately included in the database.

Further information about CMR acquisition, sequences and quantification can be consulted elsewhere<sup>12,13</sup> or in the [supplementary data](#).

The interobserver variability for the calculation of traditional CMR indexes used in the present study in our laboratory has been previously reported and is less than 5%.<sup>12,13</sup>

## Endpoints and follow-up

CMR-derived MVO at 1 week was selected as the primary endpoint of this study.

The secondary endpoints were the relationship between EpCAM measured at 24 hours after percutaneous coronary intervention and long-term ventricular remodeling, as reflected by 6-month left ventricular (LV) ejection fraction (LVEF) and LV end-systolic volume index.

## Statistical analysis

Data were tested for normal distribution using the Kolmogorov–Smirnov test. Continuous normally distributed data are expressed as the mean  $\pm$  standard deviation of the mean and were compared using paired and unpaired Student *t* tests. Nonparametric data are expressed as the median [interquartile range] and were compared using the Mann-Whitney *U* test. Group percentages were compared using the chi-square test or the Fisher exact test where appropriate.

A multivariate logistic regression analysis with the forward conditional stepwise method was performed to test the association of baseline characteristics with the presence of MVO at 1 week in the entire study group. All input variables were entered

**Table 1**

Baseline characteristics, angiographic indices, and biomarkers of the entire cohort and of patients with EpCAM above or below the median

	All patients	EpCAM below median (< 4.48 pg/mL)	EpCAM above median (> 4.48 pg/mL)	<i>P</i>
<b>Number of patients</b>	106	53	53	
<b>Baseline characteristics</b>				
Age, y	59 $\pm$ 13	58 $\pm$ 14	60 $\pm$ 12	.303
Male sex, %	81 (76)	46 (87)	35 (66)	.012
Diabetes mellitus, %	22 (21)	13 (25)	9 (17)	.338
Hypertension, %	55 (52)	28 (53)	27 (51)	.846
Dyslipidemia, %	48 (45)	20 (38)	28 (53)	.119
Smoker, %	66 (62)	31 (59)	35 (66)	.423
Killip class, %				.549
1	86 (81)	44 (83)	42 (79)	
2	15 (14)	8 (15)	7 (13)	
3	1 (1)	0 (0)	1 (2)	
4	4 (4)	1 (2)	3 (6)	
Time to reperfusion, min	200 [145-390]	188 [124-413]	210 [150-329]	.827
GRACE risk score	133 $\pm$ 35	130 $\pm$ 34	137 $\pm$ 37	.286
TIMI risk score	2 [1-4]	2 [1-3]	3 [1-4]	.433
<b>Angiographic indices</b>				
Anterior infarction, %	54 (51)	28 (53)	26 (49)	.698
Multivessel disease, %	32 (60)	15 (28)	17 (32)	.609
TIMI flow grade before PCI, %				.248
0	66 (62)	38 (72)	28 (53)	
1	6 (6)	2 (4)	4 (8)	
2	7 (7)	2 (4)	5 (9)	
3	27 (25)	11 (21)	16 (30)	
TIMI flow grade after PCI, %				.574
0	0 (0)	0 (0)	0 (0)	
1	1 (1)	0 (0)	1 (2)	
2	9 (8)	5 (10)	4 (8)	
3	96 (91)	48 (90)	48 (90)	
<b>Biomarkers</b>				
hs-TnT	2807 [1433-5140]	2898 [1706-5429]	2769 [624-3979]	.184
Maximum eosinophil count ( $\times 10^3$ cells/mL)	200 [157-300]	200 [140-300]	220 [165-305]	.286
Minimum eosinophil count ( $\times 10^3$ cells/mL)	30 [10-80]	30 [15-75]	30 [10-80]	.954
Maximum lymphocyte count ( $\times 10^3$ cells/mL)	2400 [1905-3495]	2320 [1920-3410]	2510 [1900-3515]	.315
Minimum lymphocyte count ( $\times 10^3$ cells/mL)	1260 [970-1612]	1290 [990-1635]	1210 [940-1590]	.501
EpCAM (pg/mL)	4.5 $\pm$ 1.0	3.7 $\pm$ 0.5	5.3 $\pm$ 0.6	< .001

EpCAM, epithelial cell adhesion molecule; GRACE, Global Registry of Acute Coronary Events; hs-TnT, high-sensitive troponin T; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Data are expressed as No. (%), mean  $\pm$  standard deviation, or median [range].

simultaneously to obtain the odds ratio (OR), 95% confidence interval (95%CI) for the variables not independently associated with MVO. For the multivariate regression analysis, all baseline characteristics were chosen. EpCAM was the only index independently associated with the presence of MVO at 1 week.

The association between EpCAM and CMR indices was performed using the Pearson or Spearman correlation test were appropriate.

Discrimination accuracy (c-statistic) for MVO prediction was computed for the EpCAM.

The relationship between EpCAM (pg/mL) and 1-week MVO (% of LV mass), between EpCAM (pg/mL) and 1-week infarct size (% of LV mass), and between EpCAM (pg/mL) and 1-week intramyocardial hemorrhage (number of segments) were analyzed using multivariate linear regression models in the entire study group. For the multivariate regression analyses, all baseline characteristics were chosen as covariates for the adjustment. Next, a reduced and parsimonious model was derived using backward stepwise selection.

The covariates included in the final model for 1-week infarct size were anterior infarction, Thrombolysis in Myocardial Infarction (TIMI) flow grade before percutaneous coronary intervention, high-sensitivity troponin T, and lymphocyte minimum count. For 1-week MVO, TIMI flow grade before PCI and high-sensitivity troponin T were selected in the final model. In the case of 1-week intramyocardial hemorrhage, diabetes mellitus, TIMI flow grade after percutaneous coronary intervention, anterior infarction and time to reperfusion were included in the final model.

The relationship at 6 months between EpCAM (pg/mL) and LVEF (%) and between EpCAM (pg/mL) and LV end-systolic volume index (mL/m<sup>2</sup>) were analyzed using multivariate linear regression models. All baseline characteristics were chosen as covariates for the adjustment. Next, a reduced and parsimonious model was derived using backward stepwise selection.

The covariates included in the final model for LVEF at 6 months were anterior infarction, TIMI flow grade before percutaneous

coronary intervention, minimum lymphocyte count and high-sensitivity troponin T. In the case of 6-month LV end-systolic volume index, minimum lymphocyte count and high-sensitivity troponin T were included in the final model.

Statistical significance was considered for a 2-tailed  $P < .05$ . The SPSS statistical package (version 15.0, SPSS Inc., Chicago, Illinois) and STATA (Version 9.0, StataCorp, College Station, Texas) were used throughout.

## RESULTS

We prospectively enrolled 127 consecutive patients discharged between July 2013 and December 2017 with inclusion criteria. Exclusion criteria were death ( $n = 2$ ), reinfarction ( $n = 4$ ), or clinical instability ( $n = 4$ ) during the first 6 months postdischarge or any contraindication to CMR ( $n = 11$ ). The final study group comprised 106 STEMI patients. The study patient flowchart is presented in [figure 1 of the supplementary data](#).

The mean age of the sample was  $59 \pm 13$  years, and 81 (76%) were men. The infarct location was anterior in 54 patients (51%), and 20 patients (19%) were in Killip class  $\geq$  II. At 1-week CMR, the median MVO was 0 [0-2.3] % of LV mass and the mean LVEF was  $53 \pm 11\%$ .

### Association of EpCAM with extent of 1-week MVO

Serum levels of EpCAM were quantified 24 hours after reperfusion, with a median EpCAM of 4.48 pg/mL. The baseline characteristics of patients with EpCAM values below and above median are presented in [table 1](#), showing a higher number of male than female patients with circulating EpCAM below the median.

For the CMR imaging variables at 1 week, patients with below median EpCAM levels displayed greater MVO ( $0.7 [0-4.2]$  vs  $0 [0-1.5]$ ,  $P = .021$ ), infarct size ( $24 \pm 15$  vs  $17 \pm 12$ ,  $P = .019$ ), and intramyocardial hemorrhage ( $0 [0-2]$  vs  $0 [0-0.8]$ ,  $P = .010$ ) ([table 2](#)).

**Table 2**  
CMR characteristics of the entire cohort and of patients with EpCAM above or below the median

	All patients	EpCAM below median (< 4.48 pg/mL)	EpCAM above median (> 4.48 pg/mL)	P
<b>Number of patients</b>	106	53	53	
<b>1-week CMR</b>				
LVEF, %	$53 \pm 11$	$51 \pm 12$	$55 \pm 11$	.087
LV end-diastolic volume index, mL/m <sup>2</sup>	$77 \pm 17$	$77 \pm 18$	$76 \pm 17$	.810
LV end-systolic volume index, mL/m <sup>2</sup>	$37 \pm 14$	$38 \pm 14$	$36 \pm 15$	.399
LV mass, g/m <sup>2</sup>	$78 \pm 15$	$79 \pm 16$	$76 \pm 13$	.195
Edema (% of LV mass)	$30 \pm 16$	$33 \pm 15$	$28 \pm 16$	.088
MVO (% of LV mass)	0 [0-2.3]	0.7 [0-4.2]	0 [0-1.5]	.021
Infarct size (% of LV mass)	$20 \pm 14$	$24 \pm 15$	$17 \pm 12$	.019
Myocardial salvage index, %	$31 \pm 28$	$30 \pm 27$	$33 \pm 29$	.649
Intramyocardial hemorrhage (No. of segments)	0 [0-2]	0 [0-2]	0 [0-0.8]	.010
<b>6-month CMR</b>				
LVEF, %	$58 \pm 12$	$55 \pm 13$	$61 \pm 10$	.009
LV end-diastolic volume index, mL/m <sup>2</sup>	$76 \pm 20$	$77 \pm 23$	$74 \pm 17$	.414
LV end-systolic volume index, mL/m <sup>2</sup>	$33 \pm 17$	$36 \pm 20$	$30 \pm 13$	.043
LV mass, g/m <sup>2</sup>	$71 \pm 17$	$71 \pm 16$	$71 \pm 17$	.957
MVO (% of LV mass)	0 [0-0]	0 [0-0]	0 [0-0]	.658
Infarct size (% of LV mass)	$17 \pm 11$	$17 \pm 12$	$17 \pm 10$	.885

CMR, cardiovascular magnetic resonance; EpCAM, epithelial cell adhesion molecule; LV, left ventricular; LVEF, left ventricular ejection fraction; MVO, microvascular obstruction.

The data are expressed as absolute numbers, mean  $\pm$  standard deviation, or median [range].

**Table 3**  
Predictors of 1-week MVO: univariate and multivariate analysis

	Univariate analysis		Multivariate analysis	
	Odds ratio [95%CI]	P	Odds ratio [95%CI]	P
Age, y	0.99 [0.96-1.02]	.404	0.95 [0.88-1.03]	.678
Male sex, %	0.78 [0.31-1.96]	.605	0.42 [0.10-1.81]	.181
Diabetes mellitus, %	2.07 [0.79-5.47]	.141	3.18 [0.56-18.03]	.263
Hypertension, %	1.65 [0.76-3.56]	.205	1.09 [0.31-3.85]	.481
Dyslipidemia, %	0.76 [0.35-1.65]	.488	1.13 [0.33-3.85]	.892
Smoker, %	0.83 [0.38-1.82]	.632	0.50 [0.14-1.80]	.477
Killip class, %	0.74 [0.41-1.36]	.335	0.71 [0.17-2.93]	.956
Time to reperfusion, min	1.0 [0.99-1.00]	.311	1 [0.99-1.002]	.646
GRACE risk score	1.00 [0.99-1.01]	.970	1.02 [0.99-1.05]	.501
TIMI risk score	0.96 [0.80-1.14]	.609	0.74 [0.45-1.21]	.991
Anterior infarction, %	1.52 [0.71-3.29]	.283	1.29 [0.37-4.55]	.566
Multivessel disease (%)	0.68 [0.29-1.60]	.380	0.47 [0.13-1.68]	.344
TIMI flow grade before PCI, %	0.73 [0.52-1.00]	.049	0.66 [0.42-1.04]	.057
TIMI flow grade after PCI, %	0.80 [0.25-2.52]	.702	0.64 [0.14-2.91]	.879
hs-TnT	1.00 [1.00-1.00]	.215	1 [0.99-1.01]	.339
Maximum eosinophil count (x 10 <sup>3</sup> cells/mL)	1.00 [0.99-1.00]	.788	1.003 [0.99-1.007]	.340
Minimum eosinophil count (x 10 <sup>3</sup> cells/mL)	0.99 [0.98-1.00]	.040	0.99 [0.98-1.005]	.055
Maximum lymphocyte count (x 10 <sup>3</sup> cells/mL)	1.00 [1.00-1.00]	.397	1 [0.99-1.001]	.743
Minimum lymphocyte count(x10 <sup>3</sup> cells/mL)	0.99 [0.99-1.00]	.116	1 [0.99-1.001]	.170
EpCAM, pg/mL	0.58 [0.38-0.88]	.011	0.55 [0.35-0.87]	.010

95%CI, 95% confidence interval; EpCAM, epithelial cell adhesion molecule; GRACE, Global Registry of Acute Coronary Events; hs-TnT, high-sensitive troponin T; MVO, microvascular obstruction; PCI, percutaneous coronary intervention. TIMI, Thrombolysis in Myocardial Infarction.

In a univariate analysis, EpCAM (OR, 0.58; 95%CI, 0.38-0.88;  $P = .011$ ), TIMI flow grade before percutaneous coronary intervention (OR, 0.73; 95%CI, 0.52-1.00;  $P = .049$ ) and eosinophil minimum count (OR, 0.99; 95%CI, 0.98-1.00;  $P = .040$ ) were significantly associated with the presence of MVO. However, when we included all baseline clinical variables in a multivariate logistic regression model, only EpCAM levels were independently associated with MVO (OR, 0.55; 95%CI, 0.35-0.87;  $P = .010$ ) (table 3). Moreover, EpCAM showed a significant discrimination accuracy for MVO prediction (c-statistic: 0.65; 95%CI, 0.54-0.75;  $P = .010$ , figure 1A). Baseline and CMR characteristics according to the presence of MVO at 1-week CMR are shown in table 1 of the supplementary data and table 2 of the supplementary data, respectively.

### Left ventricular remodeling at 6 months

In the chronic phase (6 months), we detected an increase in LVEF ( $58 \pm 12$  vs  $53 \pm 11$ ,  $P < .001$ ), and a decrease in LV end-systolic volume index ( $33 \pm 17$  vs  $37 \pm 14$  mL/m<sup>2</sup>,  $P = .007$ ) and infarct size ( $17 \pm 11$  vs  $20 \pm 14\%$ ,  $P = .001$ ) compared with baseline characteristics. Conversely, no difference was observed in mean LV end-diastolic volume index ( $76 \pm 20$  vs  $77 \pm 17$  mL/m<sup>2</sup>,  $P = .932$ ). MVO and intramyocardial hemorrhage had almost vanished in all patients at 6 months.

For the CMR imaging variables at 6 months, patients with EpCAM levels below the median displayed lower values of LVEF ( $55 \pm 13$  vs  $61 \pm 10$ ,  $P = .009$ ) and higher values of LV end-systolic volume index ( $36 \pm 20$  vs  $30 \pm 13$ ,  $P = .043$ ) (table 2). LVEF at 6 months (OR, 1.05; 95%CI, 1.01-1.09,  $P = .012$ ) and LV end-systolic volume index at 6 months (OR, 0.97; 95%CI, 0.95-0.99;  $P = .048$ ) were significantly associated with low EpCAM levels.

### Adjusted effect of EpCAM on short- and long-term cardiac structure and function

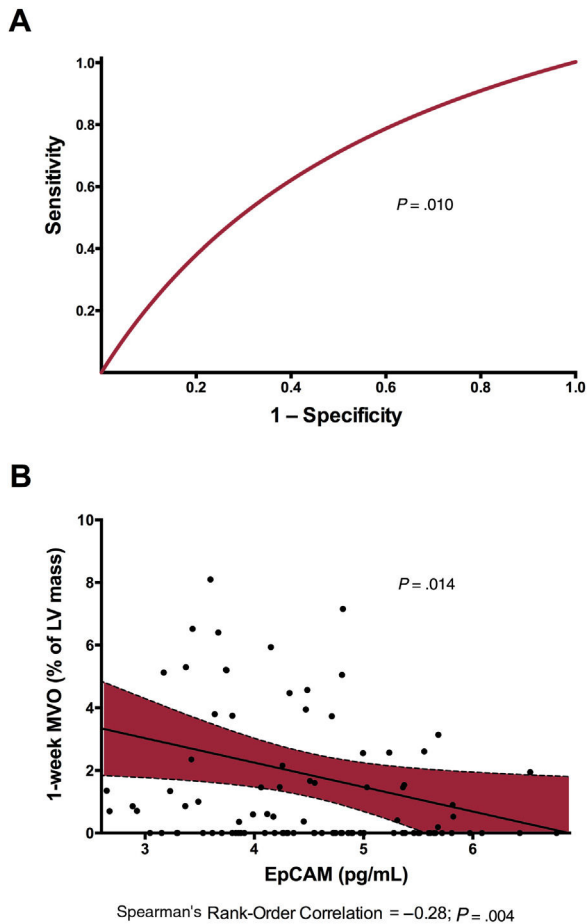
After multivariate adjustment including all baseline characteristics, serum EpCAM levels remained significantly and inversely associated with 1-week MVO ( $P = .014$ , figure 1B), 1-week infarct size ( $P = .113$ , figure 2A), and 1-week intramyocardial hemorrhage ( $P = .004$ , figure 2B). Serum EpCAM levels also showed a significant positive association with LVEF at 6 months ( $P = .017$ , figure 2C) and a nonsignificant negative association with LV end-systolic volume index at 6 months ( $P = .033$ , figure 2D).

### DISCUSSION

The main finding of our study is the association between circulating EpCAM values and CMR-derived MVO at 1 week, and long-term LV remodeling indices, in a cohort of patients with a first STEMI.

#### Microvascular obstruction

In reperfused STEMI, abnormalities in microcirculation can occur in more than 50% of patients.<sup>1,2</sup> This phenomenon is known as MVO and brings about deleterious structural and prognostic effects such as larger infarct size, more depressed systolic function, less contractile recovery and more adverse remodeling.<sup>3,4</sup> As regards prognosis, a recent meta-analysis has demonstrated that the presence of MVO increases the 2-year risk of death, reinfarction, and heart failure.<sup>14</sup> Collectively, MVO leads to adverse structural and prognostic consequences.



**Figure 1.** Predictive value and association of EpCAM with respect to 1-week MVO. A: EpCAM showed a significant discrimination accuracy (c-statistic) for MVO prediction. B: after adjustment for baseline characteristics, EpCAM showed a negative association with 1-week MVO in the study group. EpCAM, epithelial cell adhesion molecule; LV, left ventricular; MVO, microvascular obstruction.

The last 2 decades have seen important progress in MVO detection, ranging from purely clinical predictors (Killip class, reperfusion delay, presence of diabetes mellitus and the sum of ST-segment elevation at initial electrocardiogram),<sup>5</sup> widely available markers (leukocyte counts)<sup>15,16</sup> and invasive parameters, to the advent of sophisticated noninvasive imaging techniques.<sup>1,17,18</sup> However, late gadolinium enhancement CMR is currently the most reliable technique to determine the presence and extent of MVO, defined as those regions within the core of an infarcted hyper-enhanced area with lack of contrast uptake.<sup>1,5</sup>

According to our data, minimum eosinophil count, but not Killip class, the presence of diabetes and lymphocyte count were associated with the extent of CMR-derived MVO at 1 week (table 1 of the supplementary data). Although other baseline variables have been described to correlate with the occurrence of MVO, this was not confirmed in our cohort, probably due to the limited sample size.

### Circulating biomarkers associated with microvascular obstruction

In recent years, important insights have been made into the pathophysiology and diagnosis of MVO, yet the predictive value of serum biomarkers in MVO detection and quantification is under-

studied. Some studies have reported a correlation between nonspecific biomarkers (eg, high-sensitivity troponin T, lactate dehydrogenase, and natriuretic peptides, among others) and CMR-derived MVO.<sup>19,20</sup> Although we detected a positive correlation between high-sensitivity troponin T and the extent of 1-week MVO in our cohort (table 1 of the supplementary data), this universal biomarker was not significantly associated with MVO in either the univariate or the multivariate logistic regression models. However, none of these are exclusively implicated in MVO pathophysiology. Scrutinizing biomarkers specifically related to the pathophysiology of this complication is therefore mandatory.

Although MVO is a multifactorial phenomenon with several mechanisms acting both simultaneously and sequentially in the genesis of MVO,<sup>2</sup> endothelial cells play a pivotal role. In the context of reperfused STEMI, endothelial abnormalities, specifically decreased myocardial capillary density soon after ischemia onset, augmented endothelial permeability due to sudden swelling, and disruption of endothelial cell junctions leading to interstitial edema, have been reported to play a role in deficient cardiac microvascularization post-STEMI.<sup>6,7,21</sup> Since endothelium seems to participate actively in MVO pathophysiology, our goal was to determine whether biomarkers specifically implicated in endothelial integrity are linked to the appearance of CMR-derived MVO and the resultant cardiac structure in reperfused STEMI.

### EpCAM is independently associated with MVO after reperfused STEMI

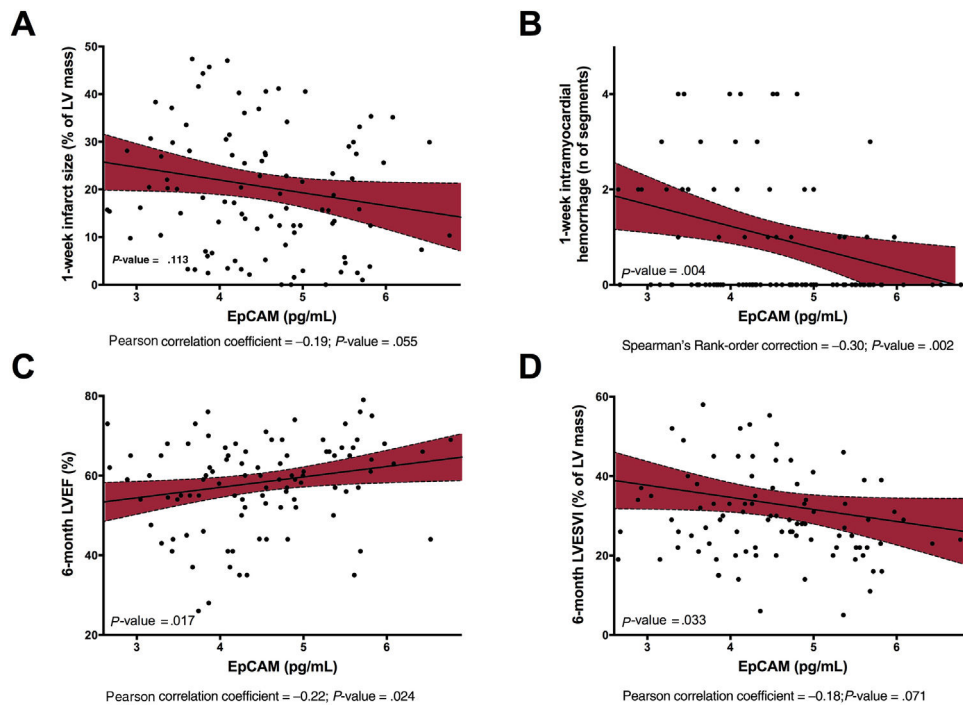
Under physiological conditions, endothelial cells control nutrients and oxygen diffusion and maintain coagulation, leukocyte migration and platelet activation under control. However, their disruption could exert deleterious effects, including edema, erythrocyte and leukocyte extravasation, as well as disturbed vascular hemostasis.<sup>22</sup> Endothelial structure and communication is mediated via tight adherens and gap junctions. EpCAM, a type I transmembrane superficial glycoprotein, is found in the basolateral membrane of multiple normal epithelial cells.<sup>8,23</sup> This protein is widely implicated in cancer development due to its overexpression in neoplastic cells. Several studies have pointed to the association of heightened EpCAM expression with metastasis in epithelial tumors and decreased patient survivorship.<sup>9</sup> Indeed, some clinical trials are currently underway in a wide variety of carcinomas to evaluate the efficacy of EpCAM-directed blocking antibodies (ie, adecatumumab or edrecolomab) as monotherapy or in combination therapy.

To our knowledge, this is the first study evaluating the role of EpCAM in the cardiovascular setting. According to our data, lower EpCAM expression correlates significantly with greater MVO at 1 week, as well as with depressed long-term systolic invasive function.

### Possible interference of EpCAM in MVO via angiogenesis promotion

Angiogenesis has been reported to participate in MVO repair. Several studies performed in both clinical and experimental models have highlighted the rapid overexpression of proangiogenic markers in both serum and infarcted myocardium.<sup>7,24–26</sup> Indeed, experimental studies aimed at promoting angiogenesis have resulted in increased microvascular density, reduced infarct size, and preserved systolic function.<sup>7,27,28</sup>

The involvement of EpCAM in promoting angiogenesis has been demonstrated in different scenarios. First, in a cohort of 98 glioma samples, EpCAM overexpression correlated with cancer malignancy and angiogenesis, as reflected by a heightened microvessel



**Figure 2.** Association of EpCAM with 1-week infarct size, 1-week intramyocardial hemorrhage, 6-month LVEF, and 6-month LVESVI. In a multivariate linear regression analysis, EpCAM showed a negative association with 1-week infarct size (A), intramyocardial hemorrhage (B) a positive relationship with 6-month LVEF (C) and 6-month LVESVI (D), after STEMI. EpCAM, epithelial cell adhesion molecule; LV, left ventricular; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end-systolic volume index; STEMI, ST-segment elevation myocardial infarction.

density.<sup>29</sup> Second, in breast cancer, EpCAM seems to promote cancer invasion since specific ablation of this molecule leads to lower vessel formation, probably due to its interference with interleukin-8.<sup>30</sup> Last, in patients with nonalcoholic steatohepatitis, a positive correlation has been found between 2 proangiogenic molecules (vascular endothelial growth factor and angiopoietin-2) and EpCAM.<sup>31</sup> According to these previous results, we could hypothesize that EpCAM may promote angiogenesis in the infarcted myocardium, resulting in decreased MVO and better ventricular remodeling. However, further research both at the bench and bedside is necessary to corroborate this issue.

Although this new biomarker could be incorporated into our daily armamentarium to early detect MVO in STEMI patients, this was not the objective of our research. Further research in a larger cohort of STEMI patients is mandatory to confirm the role of EpCAM levels on MVO appearance as well as their specific role in microvascular dysfunction.

### Study limitations

This study should be interpreted in the context of certain limitations. First, as an observational study, we cannot rule out selection bias or unmeasured confounding factors; second, the lack of serial measurements precludes assessing the significance of dynamic changes in circulating EpCAM over time. Last, infarct size, MVO, and edema were quantified by manual planimetry and there are some discrepancies about the reproducibility of this technique.

### CONCLUSIONS

Our study illustrates for the first time that circulating EpCAM values after reperfused STEMI are associated with CMR-derived MVO in the acute phases and long-term adverse ventricular

remodeling. Although further studies are necessary to corroborate the results, these findings contribute to a better understanding of MVO pathophysiology and could be helpful in biochemical assessment of the extent of MVO.

### FUNDING

This study was funded by *Instituto de Salud Carlos III* and *Fondos Europeos de Desarrollo Regional FEDER* [research grants PI17/01836, PI20/00637 and CIBERC16/11/00486, and a postgraduate contract FI18/00320 to C. Ríos-Navarro].

### AUTHORS' CONTRIBUTIONS

C. Ríos-Navarro, and J. Gavara contributed equally to this work. C. Ríos-Navarro performed research, collected the data, performed the analysis, wrote the paper, and revised the final manuscript. J. Gavara conducted research, collected the data, performed the analysis, wrote the paper, and revised the final manuscript. J. Núñez conceived and designed the analysis, collected the data, and revised the final manuscript. E. Revuelta-López conducted research, collected the data, and revised the final manuscript. J.V. Monmeneu conducted research, collected the data, and revised the final manuscript. M.P. López-Lereu conducted research, collected the data, and revised the final manuscript. E. de Dios conducted research, collected the data, and revised the final manuscript. N. Pérez-Solé conducted research, collected the data, and revised the final manuscript. J.M. Vila collected the data and revised the final manuscript. R. Oltra collected the data and revised the final manuscript. F.J. Chorro collected the data, obtained funding, and revised the final manuscript. A. Bayés-Genís conceived and designed the analysis, obtained funding, drafted the paper, and revised the final manuscript. V. Bodi conceived and

designed the analysis, obtained funding, drafted the paper, and revised the final manuscript.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

### WHAT IS KNOWN ABOUT THE TOPIC?

- MVO is associated with worse prognosis after STEMI. MVO is a multifactorial phenomenon in which endothelial cells play a pivotal role. EpCAM participates in endothelium physiology and its involvement is understudied in cardiovascular diseases. Exploring EpCAM as a predictor of extensive MVO is crucial to gain greater insight into MVO pathophysiology and provide new therapeutic alternatives.

### WHAT DOES THIS STUDY ADD?

- Circulating EpCAM values at 24 hours after coronary revascularization are negatively associated with CMR-derived MVO at 1 week. Higher EpCAM values also correlate with long-term LV remodeling indices, as reflected by a reduced LVEF and increased LV end-systolic volume. These are preliminary results and future studies are needed to confirm EpCAM as a biomarker, and eventually as a biotarget in STEMI pathophysiology.

## APPENDIX. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version, at <https://doi.org/10.1016/j.rec.2021.04.006>

## REFERENCES

- Niccoli G, Scalone G, Lerman A, Crea F. Coronary microvascular obstruction in acute myocardial infarction. *Eur Heart J*. 2016;37:1024–1033.
- Ríos-Navarro C, Marcos-Garces V, Bayes-Genis A, Husser O, Nuñez J, Bodi V. Microvascular obstruction in ST-segment elevation myocardial infarction: Looking back to move forward. *Focus on CMR J Clin Med*. 2019;28:1805.
- Hamirani YS, Wong A, Kramer CM, Salerno M. Effect of microvascular obstruction and intramyocardial hemorrhage by CMR on LV remodeling and outcomes after myocardial infarction: a systematic review and meta-analysis. *JACC Cardiovasc Imaging*. 2014;7:940–952.
- Bodi V, Sanchis J, Nuñez J, et al. Prognostic value of a comprehensive cardiac magnetic resonance assessment soon after a first ST-segment elevation myocardial infarction. *JACC Cardiovasc Imaging*. 2009;2:835–842.
- Husser O, Bodi V, Sanchis J, et al. Predictors of cardiovascular magnetic resonance-derived microvascular obstruction on patient admission in STEMI. *Int J Cardiol*. 2013;166:77–84.
- Hollander MR, de Waard GA, Konijnenberg LS, et al. Dissecting the effects of ischemia and reperfusion on the coronary microcirculation in a rat model of acute myocardial infarction. *PLoS One*. 2016;11:e0157233.
- Ríos-Navarro C, Hueso L, Miñana G, et al. Coronary serum obtained after myocardial infarction induces angiogenesis and microvascular obstruction repair. Role of hypoxia-inducible factor-1A. *Rev Esp Cardiol*. 2018;71:440–449.
- Huang L, Yang Y, Yang F, et al. Functions of EpCAM in physiology processes and diseases. *Int J Mol Med*. 2018;42:1771–1785.
- Mohtar MA, Syafruddin SE, Nasir SN, Yew LT. Revisiting the roles of pro-metastatic EpCAM in cancer. *Biomolecules*. 2020;10:255.
- Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. *Eur Heart J*. 2012;33:2251–2267.
- Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9:e95192.
- Gavara J, Rodríguez-Palomares JF, Valente F, et al. Prognostic value of strain by tissue tracking cardiac magnetic resonance after ST-segment elevation myocardial infarction. *JACC Cardiovasc Imaging*. 2018;11:1448–1457.
- Miñana G, Nuñez J, Bayés-Genis A, et al. ST2 and left ventricular remodeling after ST-segment elevation myocardial infarction: A cardiac magnetic resonance study. *Int J Cardiol*. 2018;270:336–342.
- Reichek N. Meta-analysis of MACE in MI. *What's the MO? JACC Cardiovasc Imaging*. 2014;7:953–955.
- Ríos-Navarro C, Gavara J, Vidal V, et al. Characterization and implications of the dynamics of eosinophils in blood and in the infarcted myocardium after coronary reperfusion. *PLoS One*. 2018;13:e0206344.
- Bodi V, Sanchis J, Nuñez J, et al. Post-reperfusion lymphopenia and microvascular obstruction in ST-segment elevation acute myocardial infarction. *Rev Esp Cardiol*. 2009;62:1109–1117.
- Bekkers SG, Yazdani SK, Virmani R, Waltenberger J. Microvascular obstruction: Underlying pathophysiology and clinical diagnosis. *J Am Coll Cardiol*. 2010;55:1649–1660.
- Jaffe R, Charron T, Puley G, Dick A, Strauss BH. Microvascular obstruction and the no-reflow phenomenon after percutaneous coronary intervention. *Circulation*. 2008;117:3152–3156.
- Younger JF, Plein S, Barth J, Ridgway JP, Ball SG, Greenwood JP. Troponin-I concentration 72 h after myocardial infarction correlates with infarct size and presence of microvascular obstruction. *Heart*. 2007;93:1547–1551.
- Feistritz HJ, Reinstadler SJ, Klug G, et al. Multimarker approach for the prediction of microvascular obstruction after acute ST-segment elevation myocardial infarction: a prospective, observational study. *BMC Cardiovasc Disord*. 2016;16:239.
- Yajima S, Miyagawa S, Fukushima S, et al. Microvascular dysfunction related to progressive left ventricular remodeling due to chronic occlusion of the left anterior descending artery in an adult porcine heart. *Int Heart J*. 2019;60:715–727.
- Jackson SP, Darbousset R, Shoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood*. 2019;133:906–918.
- Schnell U, Cirulli V, Giepmans BNG. EpCAM: Structure and function in health and disease. *Biochim Biophys Acta*. 2013;1828:1989–2001.
- Liu KL, Lin SM, Chang CH, Chen YC, Chu PH. Plasma angiotensin-1 level, left ventricular ejection fraction, and multivessel disease predict development of 1-year major adverse cardiovascular events in patients with acute ST elevation myocardial infarction – a pilot study. *Int J Cardiol*. 2015;182:155–160.
- Iribarren C, Phelps BH, Darbinian JA, et al. Circulating angiotensins-1 and -2, angiotensin receptor Tie-2 and vascular endothelial growth factor-A as biomarkers of acute myocardial infarction: a prospective nested case-control study. *BMC Cardiovasc Disord*. 2011;11:31.
- Hashimoto E, Ogita T, Nakaoka T, Matsuoka R, Takao A, Kira Y. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. *Am J Physiol*. 1994;267:H1948–H1954.
- Siddiqui AJ, Blomberg P, Wårdell E, et al. Combination of angiotensin-1 and vascular endothelial growth factor gene therapy enhances arteriogenesis in the ischemic myocardium. *Biochem Biophys Res Commun*. 2003;310:1002–1009.
- Ríos-Navarro C, Hueso L, Diaz A, et al. Role of antiangiogenic VEGF-A 165 b in angiogenesis and systolic function after reperfused myocardial infarction. *Rev Esp Cardiol*. 2021;74:131–139.
- Chen X, Ma WY, Xu SC, et al. The overexpression of Epithelial cell adhesion molecule (EpCAM) in glioma. *J Neurooncol*. 2014;119:39–47.
- Sankpal NV, Fleming TP, Gillanders WE. EpCAM modulates NF-κB signaling and interleukin-8 expression in breast cancer. *Mol Cancer Res*. 2013;11:418–426.
- Siddiqui H, Rawal P, Bihari C, Arora N, Kaur S. Vascular endothelial growth factor promotes proliferation of epithelial cell adhesion molecule-positive cells in non-alcoholic steatohepatitis. *J Clin Exp Hepatol*. 2020;10:275–283.