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Impact of depression on circulating endothelial progenitor cells in patients with acute coronary syndromes:a pilot study

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Abstract:	Aims. Depression has been identified as a risk factor for an adverse prognosis and reduced survival in patients with acute coronary syndrome (ACS). The number of endothelial progenitor cells (EPCs) is an independent predictor of clinical outcomes in patients with ACS. The aim was to evaluate the impact of depression on EPC levels in patients with ACS. Methods. Out of 74 ACS patients (23 non-ST-segment elevation myocardial infarction (NSTEMI), 48 STEMI), 36 had a diagnosis of major depressive episode (MDE) according to DSM-IV criteria at the time of the inclusion in the study. Control groups were: 15 healthy subjects and 18 patients with current MDE without a history of

	cardiovascular diseases. EPCs were defined as CD34+CD133+KDR+ and evaluated by flow cytometry. All patients underwent standardized cardiological and psychopathological evaluations. Parametric and non-parametric statistical tests were performed where appropriate. Results. ACS patients with MDE showed a significant decrease in circulating EPC number compared to ACS patients without MDE ($P<0.001$). The ACS study population was then subdivided into STEMI and NSTEMI groups, and inside each group again patients with MDE showed a significant decrease in circulating CD34+CD133+KDR+ EPCs compared to others ($P<0.001$). Conclusions. We showed that ACS patients with MDE have a reduced number of circulating CD34+CD133+KDR+ cells compared to ACS patient without MDE, suggesting that the presence of MDE reduce the response of bone-marrow to acute ischemic events. Considering the reparative role of EPCs in ACS patients, we suppose that patients with MDE might be protected less than patients without MDE.

Original Article

Impact of depression on circulating endothelial progenitor cells in patients with acute coronary syndromes:a pilot study

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Introduction

There is increasing knowledge regarding comorbidity between depression and cardiovascular disease, which are the most common disorders in developed countries [1-3]; indeed major depression is associated with a fourfold increase in the risk of mortality during the first 6 months after acute coronary syndrome (ACS), although the mechanisms underlying this association are still not clear [4-6]. Inflammation, for example, has been involved as a biological mechanism common to depression and ischemic heart disease and increased levels of inflammatory markers have been well documented in depression [7]. On the other hand, inflammation is thought to be involved in all stages of atherosclerosis [8], but the biological mechanisms by which inflammation can mediate the relationship between depression and increased risk of cardiovascular events have not been clarified so far.

Recently, it has been explored the effect of depressive disorder on bone marrow-derived cells described in 1997 by Asahara et al. [9]. Although a unifying definition regarding the characterization of endothelial progenitor cells (EPCs) does not exist, most researcher identify EPCs as CD34+CD133+KDR+ positive cells [10]. EPCs have the ability to influence adult vasculogenesis in areas with reduced oxygen supply or by stimulating the re-endothelialization of injured blood vessels [11-14]. A reduction in the levels of EPCs has also predictive value in progression of atherosclerotic disease, supporting the important role that endogenous vascular repair plays in modulating the clinical outcome of coronary artery disease (CAD) [15].

The relation between EPCs and cardiovascular risk factors has also been extensively investigated [15-18] and several studies gave to the number of EPCs the role of new biomarker of prognostic value, resulting independent predictor of clinical outcomes in patients with CAD [15, 19].

EPCs also appear to play an important role in the molecular background of depression-associated cardiovascular morbidity. Indeed, recent studies showed a decreased number of circulating EPCs in patients with depression compared to healthy subjects. This reduction was observed to be inversely proportional to the severity of depressive disorder and the level of tumour necrosis factor alpha (TNF-a) [20-21].

Up to now, there are no data on the relationship between circulating EPCs, depression and ACS. The objective of the present study, therefore, has been to evaluate the number of circulating EPCs in ACS patient with or without major depressive episodes (MDE), compared with healthy subjects and patients with current MDE without a history of cardiovascular diseases.

Methods

Subjects

The study population consisted of non-consecutive 74 ACS patients (55 males and 19 females, mean age 58±10 years) who were admitted to the Intensive Care Units of three Cardiology Units (La Spezia, Sarzana and Lucca) serving the same catchment area. Patients were admitted if they met the criteria for ACS, either acute myocardial infarct

with or without ST-segment elevation (STEMI and NSTEMI, respectively) or unstable angina (UA), verified by standard ACS criteria [22].

Exclusion criteria were age > 79 years, neoplastic disease, severe pulmonary, hepatic or renal insufficiency, infections requiring antibiotics, autoimmune disease, immunosuppressive therapy, severe anaemia and severe degenerative disease of the central nervous system.

Control groups included: 18 psychiatric patients with current MDE without cardiovascular disorders, enrolled from the Department of Psychiatry of the University of Pisa and 15 healthy volunteers ascertained from personnel working at the Cardiothoracic and Vascular Department of University of Pisa, evaluated by the Structured Clinical Interview for Psychiatric Disorders (SCID-I) [23], to determine the presence of Axis I psychiatric disorders according to DSM-IV criteria. Both control groups were matched for gender, age, and smoking status with ACS patients sample. Exclusion criteria were the same as those for ACS patients. The study was conducted in accordance with the Declaration of Helsinki, was approved by the local ethics committees and written informed consent was obtained from all patients (or their guardians).

The study complied with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations [24].

Study design

Complete cardiological clinical assessment and related haematological tests were performed at the time of enrolment (hospital admission for ACS event).

Psychiatric evaluations were performed as soon as patient's clinical condition allowed them, within three/four days following admission.

Blood samples collection for EPC evaluation were carried out after three/four days from ACS event. This timing was established on the basis of previous data documenting an effect of the acute phase on EPC's number [25].

Cardiovascular risk factors, including tobacco smoking, diabetes mellitus, hypercholesterolemia, and hypertension, were determined at the time of blood collection. The body height and weight, blood pressure, and body mass index (BMI) of all subjects were measured. Hypertension was defined as either resting systolic or diastolic blood pressure $\geq$ 140/90mmHg at two different times or on medications. Diabetes mellitus was defined as a serum fasting glucose of $\geq$ 7.1 mmol/l or on medications. Hypercholesterolemia was defined as a fasting total serum cholesterol level of $\geq$ 4.9 mmol/l or on medications. Smoking status was recorded as current smoker or past smoker. Based on the observation of Kondo et al. [26] on the effects of smoking cessation on EPC levels, ex-smokers were defined as those who had quit smoking at least 1 month before the cardiovascular events. Fasting blood samples were obtained from all subjects to determine serum creatinine, glucose, C-reactive protein (CRP) level and lipid levels.

Cardiological evaluation

Data on patients' demographics, medical history, clinical characteristics, electrocardiographic findings, ACS definition and treatment interventions, as well as in-hospital outcomes were reported in a detailed standardized case record form, adapted from the Blitz Study [27], by a trained cardiologist. The details on symptoms onset, first medical help seeking and arrival at hospital were collected as soon as patients could be interviewed. Particular care was taken in assessing the timing of hospital arrival, ECG execution, and reperfusion treatment. Additional items included length of stay in the CCU and overall hospital stay, timing of invasive procedures and transfer to a tertiary care hospital to undergo coronary angiography and/or revascularization.

Psychiatric Assessment

All patients were evaluated by the SCID-I [23] to determine the presence of Axis I psychiatric disorders according to DSM-IV criteria. The presence and severity of depression was assessed by the 17-Item Hamilton Depression Rating Scale (HDRS) [28]. The Hamilton Depression (HAM-D) Rating Scale provides an indication of depression and, over time, a guide to recovery. It is one of the most widely used and accepted outcome measures for evaluating the severity of depression symptoms. The HAM-D was administered by a trained psychiatrist or psychologist, blind to psychiatric MDE diagnosis, using a semi-structured interview. Eight items are scored on a 5-point scale, ranging from 0 = not present to 4 = severe. Nine are scored from 0-2. Based on the

HDRS-17 scale, the American College of Neuropsychopharmacology (ACNP) recommended using scores of ≤ 5 to define the absence of clinically relevant depression [29]. Scores between 6 and 13 indicate mild depression, from 14 to 18 moderate depression, from 19 to above severe depression.

Evaluation of circulating endothelial progenitor cells

The circulating EPCs were measured after 3-4 day of ACS event and defined by the expression of surface markers CD34/KDR/CD133 by flow cytometric analysis [30-31]. EDTA-peripheral blood samples were processed within 4 h after collection. Briefly, 100 µl of samples of peripheral venous blood of controls and ACS patients were incubated with PerCP-conjugated monoclonal antibodies anti human CD34, AlexaFluor 647-conjugated anti KDR and PE- conjugated anti CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany) in the dark at 4°C, according to manufacturers' instruction. Red cells were lysed with 1x BD FACS Lysis solution (BD Pharmigen, UK) and incubated for 5 minutes at room temperature. A PE isotype-matched antibody were used as negative control.

Validation of the assay was performed adopting the gating strategy defined by the International Society of Haematotherapy and Graft Engineering (ISHAGE) guidelines [32]. Quantitative fluorescence analysis were performed with a FACS-Calibur instrument and data were processed using Cell Quest Software (BD Pharmigen, UK). Each analysis

included at least 500,000 events. The number of EPCs was expressed as absolute number of cells per ml of blood.

Statistical analysis

Continuous data were expressed as mean ± SD. The normality of distributions of EPC numbers was assessed using a one-sample Kolmogorov-Smirnov test. As EPCs resulted non-normal distributed data were log-transformed, and after normalization, comparisons between more than two groups were performed by one-way ANOVA. Continuous variables were compared with Student's t-test or Mann–Whitney *U*-test, as appropriate. Categorical data were compared using Fishers' exact probability or χ^2-test, as appropriate. In addition, McNemar's tests were performed in 2x2 contingency tables for matched pairs of subjects to evaluate marginal homogeneity. The Spearman's test was used to identify significant correlations between two numerical variables. Statistical significance was taken at the two-tailed $p<0.05$ level. All analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20 (SPSS, Chicago, IL, USA).

Results

Baseline Characteristics

The 74 (55 males and 19 females) adult ACS patients were sub-typed as follows: n=48 STEMI (age 58±11 years); n=23 NSTEMI (age 60±7 years); n=3 UA (age 62±13 years). Patients' characteristics at baseline, including clinical risk factors and therapy, are summarized in Table 1.

There were no significant differences in age, gender and prevalence of risk factors between the ACS patients with or without MDE (Table 1; all P>0.05). Furthermore, there were no significant differences in BMI, serum total cholesterol, low and high density lipoprotein and triglycerides, creatinine, CRP, and fasting blood sugar levels between the two groups (all P>0.05; table 1).

Evaluation of depression in ACS patient

Of 74 ACS patients, 36 (n=22 STEMI, age 57± 12 years; n=12 NSTEMI, age 61±6 years; n=2 UA, age 69±1 years) resulted to have a MDE according to the SCID-I. The 36 ACS patients with MDE reported a mean HDRS total score of 7.9 ± 5. Demographic and clinical characteristics, including laboratory tests and cardiovascular risk factors, of ACS patient with or without MDE, are summarized in Table 1.

EPC levels in peripheral blood samples of patients with ACS

As shown in Figure 1, ACS patients with MDE (ACS + MDE) had a significant decrease of circulating CD34+CD133+KDR+ EPC levels compared to ACS patients without MDE (ACS) ($P<0.001$). Moreover, EPC levels was significantly reduced in patients with current MDE without a history of cardiovascular disease, here defined as cDE (psychiatric controls) compared to healthy subjects ($P<0.001$). Finally, EPC levels was significantly reduced in ACS patients without MDE (ACS) compared to healthy subjects (Ctr) ($P<0.05$).

The change in EPC numbers correlated negatively with HDRS in patients with ACS ($r = - 0.34$, $P= 0.0028$) (Figure 2).

EPC levels in STEMI and NSTEMI patients

The study population was then analyzed according to STEMI and NSTEMI groups. As show in Figure 3(A), STEMI patients with MDE (STEMI + MDE) showed a significant decrease in circulating CD133+CD34+KDR+ EPC numbers compared to patients without MDE (STEMI) ($P<0.001$). Again, CD133+CD34+KDR+ EPC levels were reduced in STEMI patients without MDE (STEMI) compared to healthy subjects (Ctr) ($P<0.05$) and between healthy subjects and cDE ($P<0.001$).

Figure 3 (B) shows log transformed value of CD133+CD34+KDR+ EPC numbers in NSTEMI patients. NSTEMI patients with MDE showed a significant decrease in CD133+CD34+KDR+ cell numbers compared to NSTEMI patients without MDE

(P<0.05). However, in this group there was no significant difference in EPC levels between NSTEMI patients without MDE and healthy subjects (P>0.05).

Discussion

Several clinical studies have shown that the number of circulating EPCs is inversely proportional to risk factors for atherosclerosis, endothelial dysfunction and future cardiovascular events [15, 18]. Dome et al., as well as Yang et al., have indicated that EPC counts are decreased in patients without specific cardiovascular risk factors with a current episode of depression [20, 33]. Moreover, Friedrich et al. [34], reported that CD133+CD34+KDR+ cells (which have a frequency in peripheral blood similar to CD34+KDR+ cells) are more vasoregenerative and represent a more immature EPC phenotype, which further matures into endothelial cells. On these assumptions, we explored the level of circulating CD133+CD34+KDR+ EPCs in patients with recent ACS with concomitant clinically depression as compared to those without. Our data showed that the presence of depression was associated with a significantly impaired release of circulating CD133+CD34+KDR+ cells in ACS patients ($P<0.001$).

It seems worth to emphasize that there were no significant differences in age, gender and prevalence of risk factors between the ACS patients with or without depression. Furthermore, there were no significant differences in BMI, serum total cholesterol, low and high-density lipoprotein and triglycerides, creatinine, CRP, and fasting blood sugar levels between the two groups. Several studies reported that in ACS patients, there seems to be a trend toward an elevated level of EPCs, suggesting that these cells are possibly mobilized in an attempt to participate in vessel repair after severe ischemia [25, 35-36]. According to Grundmann et al. [37], cell count increases after 3-4 day of an ACS

event, however we observed that the number of cells does not reach the value observed in healthy volunteers. The increased CD133+CD34+KDR+ cell levels in ACS patients may be due to the mobilization of cells from bone-marrow caused by acute vascular injury.

We also found that cells were significantly reduced in ACS patients with current MDE compared to ACS patients without MDE ($P<0.001$). Moreover, according to data obtained by Dome et al. [20], we found that cells were significantly reduced in psychiatric controls compared to healthy subjects ($P<0.001$). These data indicate that depression itself, independently of its association with an acute coronary syndrome, has an impact on circulating EPCs. In fact, the evaluation of severity of depression by HDRS embraces a period of time extended from a week preceding the acute cardiac event to the moment of evaluation. Baseline psychiatric evaluation occurred within the first three days from admission; therefore, it is more likely that the cardiac event, rather than acute setting per se, had a relationship with onset of depression. The causal relationships between these two phenomena are far from being clear. Dome et al. [20] have hypothesized a relationship with chronic inflammation processes. These authors, in fact, found a decreased level of circulating EPCs in patients with a current episode of depression and a significantly elevated TNF-a concentration. Moreover, they observed a significant inverse correlation between TNF-a and EPC levels.

Finally, we observed a significant decrease of CD133+CD34+KDR+ EPC levels in patients with MDE compared to ACS patients without MDE, independently on whether

they had a STEMI or a NSTEMI. However, no significant differences in cell levels were observed between the NSTEMI subgroup without concomitant MDE and healthy subjects ($P > 0.05$). These data corroborate the notion that the recruitment of progenitor cells is related to the duration of ischemia rather than to the size of the ischemic myocardial area [38].

Limitations

Some limitations of our study must be acknowledged. First, the relatively small number of patients enrolled may limit generalizability of our results. Second, NSTEMI patients were relatively underrepresented in our sample due to the exclusion criteria of 80 years.

Conclusions

This is the first study, to the best of our knowledge, showing that ACS patients (both STEMI and NSTEMI) with concomitant clinically relevant depressive symptoms have a reduced level of circulating CD133+CD34+KDR+ EPCs. These data suggest that the presence of depression affects bone-marrow response to acute ischemic event. Considering the reparative role of EPCs in ACS patients, it can be hypothesized that ACS patients who are also depressed may be less protected than patients without MDE from the risk of occurrence of new major cardiac events. Notwithstanding, prospective studies are needed to confirm this hypothesis. From such a viewpoint, we are conducting a prospective study to explore the strength of association between presence of depression, EPC levels and cardiac outcome in patients with ACS.

Finally, our study suggests that individuals with cardiovascular risk factors or with definite cardiovascular diseases, such as ACS, should be screened for depression.

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The authors declare no conflict of interests.

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Figure legends

Figure 1. EPC levels in ACS patients. CD133+CD34+KDR+ EPCs levels are expressed as box plots displaying medians (lines), interquartile range (boxes) and maximum and minimum values (whiskers), of log transformed data. STEMI and NSTEMI ACS patients with major depressive episode (ACS+MDE) (n=36) show a reduced level of circulating EPCs compared with STEMI and NSTEMI ACS patients without MDE (ACS) (n=38), ***P<0.001. Moreover, psychiatric controls (cDE) (n=18) show a reduced EPC levels compared with healthy subjects (Ctr) (n=15), ***P<0.001. Change in the groups were compared using one-way ANOVA and Newman-Keuls multiple comparison post-hoc test (p<0.05).

Figure 2. Relationship between progenitor cell levels (EPCs) and Hamilton Depression Rating Scale (HDRS) in ACS patients. (r = - 0.34, P<0.005)

Figure 3. EPC levels in STEMI (A) and in NSTEMI (B) patients. CD133+CD34+KDR+ EPCs levels are expressed as box plots displaying medians (lines), interquartile range (boxes) and maximum and minimum values (whiskers) of log transformed data. STEMI patients (**A**) with major depressive episode (STEMI +MDE) (n=23) show a reduced level of circulating EPCs compared with STEMI patient without MDE (STEMI) (n=25), ***P<0.001. NSTEMI patients (**B**) with MDE (NSTEMI +MDE) (n=12) show a reduced EPC levels compared with NSTEMI patient without MDE (NSTEMI) (n=14), *P<0.05. Change in the groups were compared using one-way ANOVA and Newman-Keuls multiple comparison post-hoc test (p <0.05).

Figure 1
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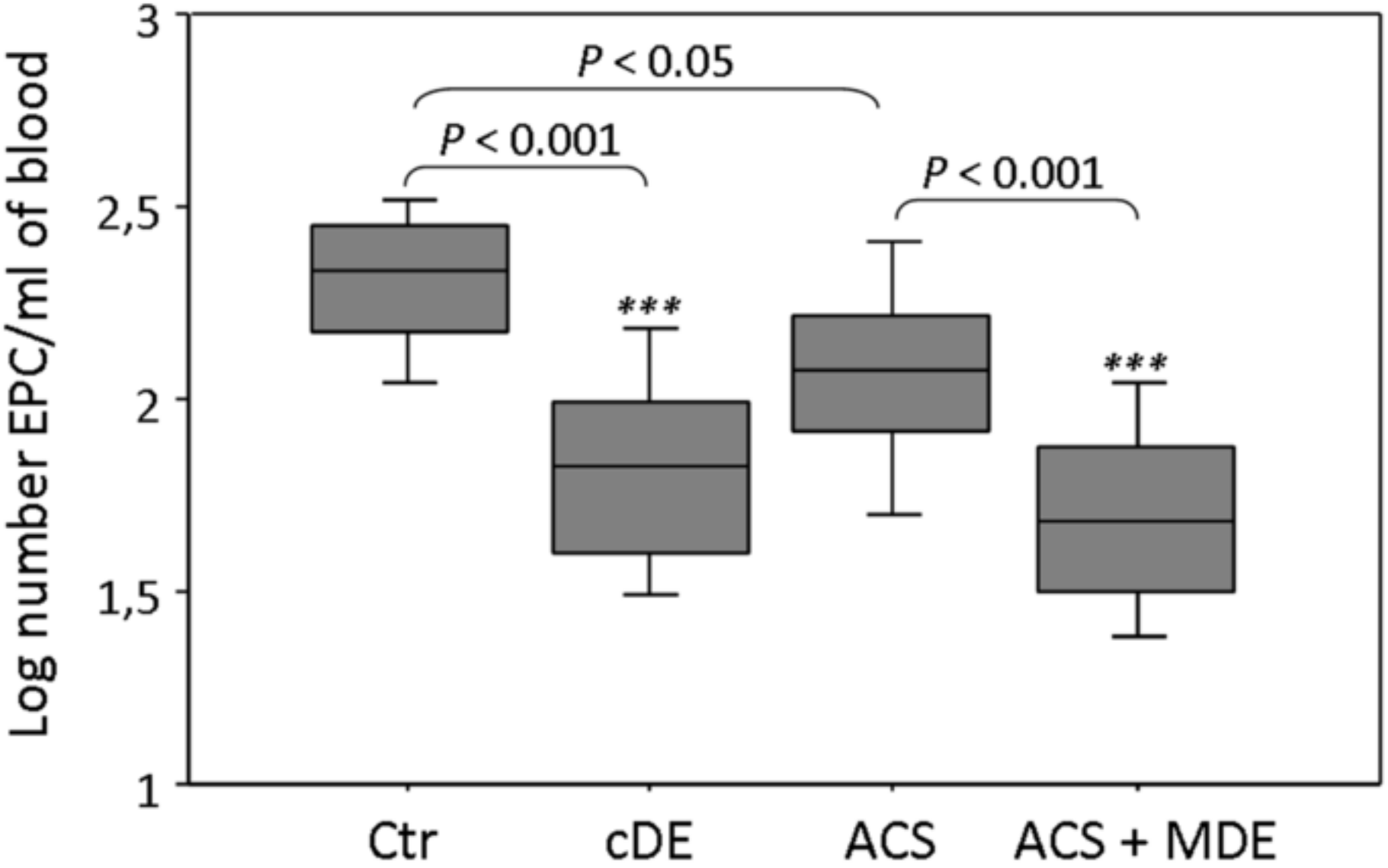

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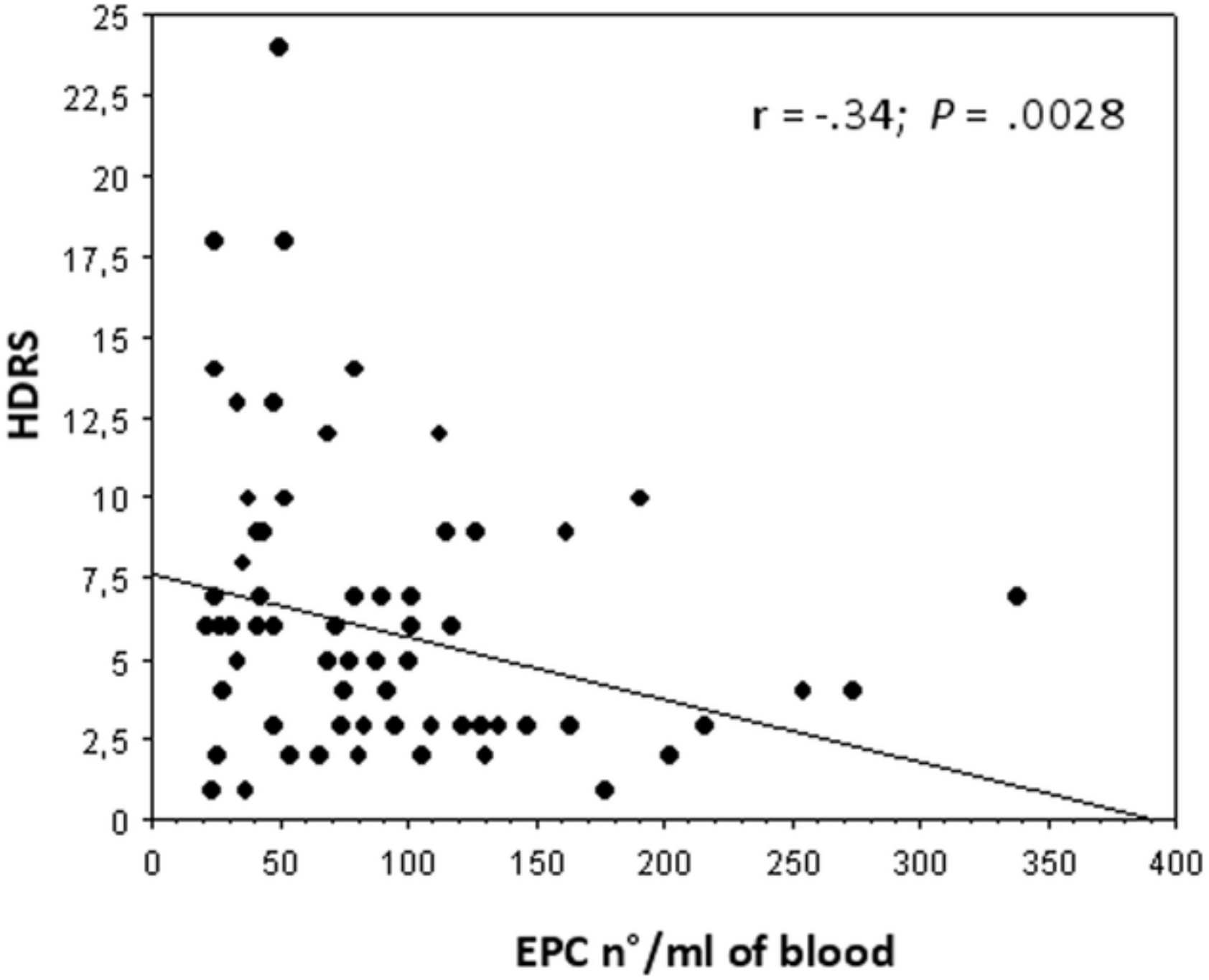

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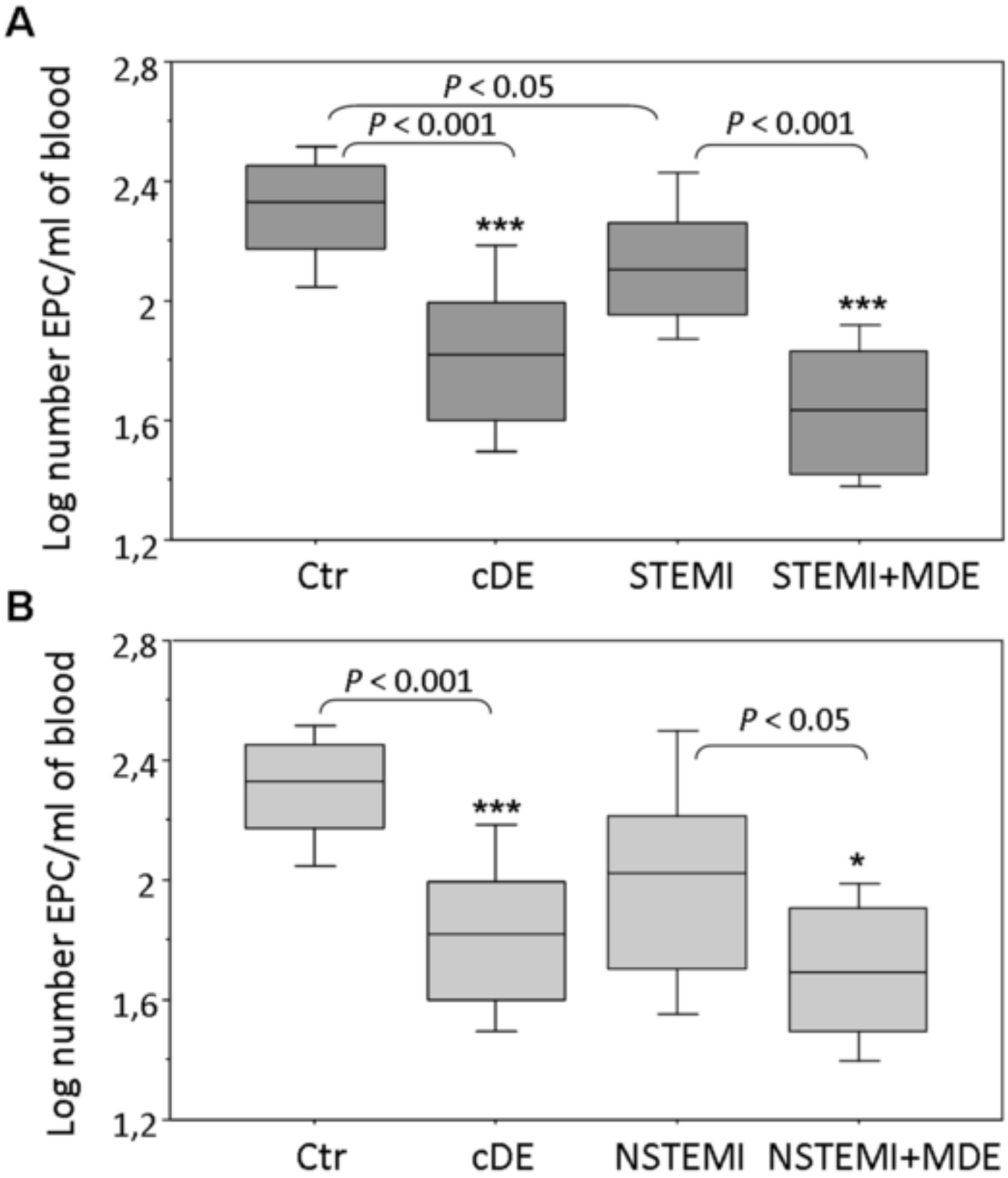

Table 1. Patients' baseline characteristics

	Ctr (n=15)	cDE (n=18)	ACS (n= 38)	ACS + MDE (n=36)	P-value[#]
Age (years, mean ± SD)	49 ±12	50 ± 10	58 ± 11	59 ± 10	0.79*
Gender (male/female)	10/5	11/7	28/10 (74 vs 26 %)	27/9 (75 vs 25 %)	0.79†
STEMI, n (%)	NA	NA	26 (68 %)	22 (61 %)	0.80†
NSTEMI, n (%)	NA	NA	11 (29 %)	12 (33 %)	0.80†
UA, n (%)	NA	NA	1 (2.6 %)	2 (5.5 %)	0.80†
Cardiovascular Risk Factors					
CAD familiarity, n (%)	5 (33 %)	2 (11 %)	18 (47.4 %)	9 (25 %)	0.06†
Diabetes, n (%)	3 (20 %)	0	9 (23.7 %)	4 (11 %)	0.22†
Hypertension, n (%)	6 (40 %)	4 (22 %)	22 (57.9 %)	16 (44.4 %)	0.35†
Dyslipidemia, n (%)	1 (7 %)	4 (22 %)	19 (50 %)	20 (55.6 %)	0.65†
Active smokers, n (%)	5 (33 %)	6 (33 %)	15 (39.5 %)	15 (41.7 %)	0.40‡
Biochemical parameters					
BMI (Kg/m^2)	23 ± 4	22 ± 5	24 ± 5	23 ± 5	0.97 §
Fasting glucose (mg/dl)	86 (78-90)	82 (70-89)	103.5 (91-120)	106 (89-118)	0.85 §
Total cholesterol (mg/dl)	183 (145-195)	186 (161-202)	190 (161.7-218.7)	198 (162.7-225.7)	0.71 §
LDL cholesterol (mg/dl)	107 (96.5-118)	111 (105-121)	107 (94.5-120)	129.5 (88-139.2)	0.23 §
HDL cholesterol (mg/dl)	50 (40-66)	49 (40-56)	43 (35-51)	41.5 (39-47.5)	0.73 §
Triglycerides (mg/dl)	70 (58-99)	88 (85-117)	134.5 (94-190.2)	124 (94-163)	0.49 §
C-reactive protein (mg/l)	NA	NA	0.73 (0.31-1.69)	0.68 (0.25-0.95)	0.60 §
Creatinine (mg/dl)	0.99 (0.86-1.1)	0.82 (0.54-1.1)	0.92 (0.77-1.12)	0.93 (0.79-1.07)	0.76 §
Peak-hsTn (ng/ml)	NA	NA	6.65 (1.05-28)	5.13 (0.62-46.6)	0.64 §
Therapy at the day of EPC analysis					
Statins, n (%)	NA	NA	35 (92 %)	36 (100 %)	0.11†
ACE inhibitors, n (%)	NA	NA	5 (13 %)	4 (11 %)	0.71†
Beta-blockers, n (%)	NA	NA	34 (89 %)	34 (94 %)	>0.99†
Oral antidiabetics, n (%)	NA	NA	2 (5 %)	1 (3 %)	0.49†
Nitrates, n (%)	NA	NA	4 (11 %)	2 (5 %)	0.67†
Insulin, n (%)	NA	NA	5 (13 %)	3 (8 %)	0.71†
Diuretics, n (%)	NA	NA	7 (18 %)	7 (19 %)	>0.99†
Antiplatelet agents,n (%)	NA	NA	36 (95 %)	36 (100 %)	>0.99†
Anticoagulants, n (%)	NA	NA	7 (18 %)	6 (17 %)	0.768†

*Independent-sample t-test. †Fischer's exact test. ‡Mc Nemar test. §Mann–Whitney *U*-test. ACS, acute coronary syndrome; MDE, major depressive episode; CAD, coronary artery disease; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; hsTn, high sensitive troponine. Data are expressed as number (n), percentage (%) and as median and IQR.. [#] Difference between ACS vs ACS+MDE patients.