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# Intracoronary monocyte expression pattern and HDL subfractions after non-ST elevation myocardial infarction

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ABSTRACT

*Aims:* Coronary artery disease (CAD) is described as a range of clinical conditions including myocardial infarction (MI) and unstable angina. Lipid and apolipoprotein profiles together with the study of cholesterol deposit and efflux serve to identify novel pre and post infarct scenarios for the treatment of these patients. In (non-ST elevation myocardial infarction) NSTEMI patients, we analysed both systemic and intracoronary serum ability to accept cholesterol as well as cholesterol efflux capacity (CEC) of monocytes in terms of expression of genes involved in the reverse cholesterol transport (RCT).

*Methods and results:* While HDL-C quantity was similar between systemic and coronary arterial blood, in 21 NSTEMI patients we observed a significant reduction of the preβ-HDL fraction and the levels of Apolipoproteins AI, AII, B and E in coronary *versus* systemic serum. These data are complemented with the observed reduction of CEC. On the contrary, compared to systemic arterial monocytes, in coronary microenvironment of NSTEMI patients after myocardial infarction, the monocytes exhibited a higher mRNA expression of nuclear receptor LXRα and its targets ABCA1 and APOE, which drive cholesterol efflux capacity.

*Conclusion:* In this cross-sectional study we observe that in the immediate post infarction period, there is a spontaneous *bona fide* ligand-induced activation of the LXR driven cholesterol efflux capacity of intracoronary monocytes to overcome the reduced serum ability to accept cholesterol and to inhibit the post-infarction pro-inflammatory local microenvironment.

## 1. Introduction

Coronary artery disease (CAD) is a primary cause of death in the United States and developing countries [1]. Disorganization of the arterial intima owing to the accumulation of inflammatory cells, fatty acids, cholesterol and cellular debris is the *primum movens* in the creation of the atherosclerotic plaque [2]. The reduction of high-density lipoprotein cholesterol (HDL—C) concentration is considered a risk factor for atherosclerosis and CAD [3–5]. HDLs belong to the smallest spherical or discoidal lipoprotein fraction primarily formed by apolipoprotein A-I (apoA-I) and apoA-II [6]. HDLs can be divided into α-particles or circulating HDL and preβ-particles that represent nascent discoidal or

*Abbreviations:* CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; apoA-I, apolipoprotein A-I; RCT, reverse cholesterol transport; ABCA1, ATP-binding cassette A1; LCAT, lecithin cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; CEC, cholesterol efflux capacity; NSTEMI, non-ST elevation myocardial infarction; IPA, Ingenuity System Pathway Analysis.

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poorly-lipidated HDL [7]. HDL subclasses display different atheroprotective properties due to its protein and lipid composition [8,9] and the ability to promote the reverse cholesterol transport (RCT). RCT is a multistep process that allows the excretion of cholesterol from macrophages into the arterial wall and carries it back to the liver for the elimination through the bile and feces [10]. Macrophages expel unesterified cholesterol through passive diffusion or *via* ATP-binding cassette A1 (ABCA1) that promotes phospholipids and cholesterol efflux to lipid free apolipoproteins (ApoA-I and ApoE) and preβ-HDL [11,12]. Cholesterol into HDL is esterified by lecithin cholesterol acyltransferase (LCAT) [13] and then transferred to VLDL and LDL (rich in apolipoproteins B) to reach the liver [14]. The cholesteryl ester transfer protein (CETP) plasma protein transfers cholesteryl esters and triglycerides from the HDL fraction to other lipoprotein fractions and from the triglyceride-rich lipoproteins into the HDL fraction, respectively [15]. Furthermore, cholesteryl esters are directly transported to the liver by HDL *via* interaction with SR-BI [16].

The primary antiatherogenic mechanism of HDL is thought to be mediated by the maintenance of net cholesterol balance in the arterial wall by promoting the efflux of excess cholesterol from peripheral tissues and macrophage foam cells. The relative distribution and composition of HDL could therefore be used to define serum efficiency to accept cholesterol. In line with this, it has been demonstrated that serum ability to promote cholesterol efflux through the ABCA1 transporter is closely dependent on preβ-HDL concentration and not on HDL-C levels [17,18].

Genetic variants linked to increased HDL-C concentration did not promote beneficial vascular outcomes [19]. These observations confirm the idea that it is the quality and not quantity of HDL-C to define its atheroprotective function. A measure of HDL-C function can be assessed by the cholesterol efflux capacity (CEC) [20]. Subjects with the ApoA-$I_{Milano}$ mutation show high circulating levels of pre-β HDL and a good serum ability to remove cholesterol from macrophages despite the very low levels of HDL-C [17,21,22]. These patients display exceptional longevity due to the low number of cardiovascular events. Furthermore, CEC is inversely associated with the incidence of cardiovascular events [23] and it represents a better predictor of CAD risk than HDL-C levels [24].

In the early plaque formation stages, in the vascular endothelium differentiated macrophages can incorporate atherogenic cholesteryl ester-rich lipoprotein *via* specific surface receptors. The constant accumulation of cholesteryl ester promotes the formation of foam cells. In macrophages, cholesterol efflux is facilitated by ABCA1, ABCG1 and SR-BI transporters and this process is promoted by apolipoprotein E (ApoE). In macrophages or foam cells, cholesterol efflux is primary mediated by ABCA1, induced by the LXR/RXR axis [25]. Moreover, apoE exhibits a key function in macrophages cholesterol efflux [26]. After synthesis, ApoE is incorporated into vesicular structures and finally secreted [27]. This process promotes cholesterol efflux from macrophages inducing the production of nascent HDL particles [28].

In the present study, we analysed, for the first time, systemic and intra-coronary arterial microenvironment of Non-ST elevation myocardial infarction (NSTEMI) patients in order to evaluate the functional properties of HDL and RCT. In particular, we studied in coronary microenvironment of NSTEMI patients, serum ability to accept cholesterol together with the monocytes/macrophages ability to expel cholesterol with the aim to identify new diagnostic, preventive and therapeutic approaches.

## 2. Material and methods

### 2.1. Study population

Patient enrollment and clinical, biochemical and instrumental evaluation of myocardial infarction were carried out at the Cardiology Unit of Ospedale San Paolo (Bari, Italy). Twenty-one patients at the first diagnosis of NSTEMI (14 M:7F; mean age 63.4 ± 2.5 years), were recruited for this study. All the patients enrolled presented myocardial infarction classified type 1 by angiography. The diagnosis of myocardial infarction was assessed by the integration of typical clinical presentation, 12 lead ECG alterations (as ST depression, transient ST elevation or T-wave changes) and the significant increase of serum troponin I (TnI) levels [29]. We analysed blood from the culprit lesion: in every patient we consider culprit lesion evaluating concordance between ECG, Echocardiography and Angiography. All patients received statins (high dose) as antiplatelet therapy. The blood from coronaries was taken, during percutaneous coronary Intervention (PCI) after Angio, with the EXPORT CATHETER (Medtronic, Minneapoli US) or with ELIMINATE (Terumo, Tokyo Japan). The catheters have a syringe that can aspirate when the physician is inside the coronary: we are sure the blood was only from coronary because we opened blood flow through the Export or Eliminate only at the point of the culprit lesion and it was stopped before withdrawing the aspiration catheter from coronary.

In this population 4 patients were taking antidiabetic medications (insulin or biguanides), 11 antihypertensive drugs (ACE inhibitors, angiotensin II receptor antagonists and others), 6 high cholesterol drugs, 4 cardioaspirin. Clinical characterization of NSTEMI patients is shown in Table 1. We considered as exclusion criteria: the presence of cerebrovascular diseases, hepatic or renal and autoimmune diseases, endocrine disorders, acute or chronic systemic syndromes. We collected

**Table 1**
Clinical characterization of the study population.

| Clinical variable | NSTEMI |
|---|---|
| Age (year) | 63.4 ± 2.5 |
| Weight (kg) | 77.6 ± 2.4 |
| BMI (kg/m$^2$) | 28.4 ± 0.9 |
| WC (cm) | 98.7 ± 2.2 |
| SBP (mm Hg) | 132.4 ± 3.9 |
| DBP (mm Hg) | 76.9 ± 2.5 |
| TG (mg/dl) | 126.1 ± 13.8 |
| TC (mg/dl) | 178.1 ± 9.6 |
| HDL-C (mg/dl) | 43.8 ± 2.1 |
| LDL-C (mg/dl) | 116.4 ± 9.1 |
| Glucose (mg/dl) | 121.2 ± 12.4 |
| HbA1c (%) | 5.9 ± 0.4 |
| Insulin (μUI/ml) | 8.9 ± 2.3 |
| C-peptide (ng/ml) | 2.4 ± 0.5 |
| HOMA-IR | 2.4 ± 0.9 |
| Homocysteine (μmol/L) | 15 ± 2.9 |
| Ferritin | 192.1 ± 35.2 |
| hs-CRP (mg/L) | 15.7 ± 10.9 |
| CRP (mg/L) | 17.8 ± 10.5 |
| ESR (mm/h) | 25.5 ± 5.1 |
| GGT (U/I) | 22.4 ± 3.7 |
| AST (U/I) | 31.3 ± 5.5 |
| ALT (U/I) | 26.9 ± 5.1 |
| ALP (U/I) | 87.8 ± 12.4 |
| CK-MB (U/I) | 19.3 ± 7.3 |
| LDH (U/I) | 200 ± 26.5 |
| Tn-I (U/I) | 7.3 ± 2.2 |
| Microalbuminuria (mg/l) | 75.4 ± 60.4 |
| Uricaemia | 5.1 ± 0.3 |
| Fibrinogen (mg/dl) | 393 ± 24.2 |
| CV risk[a] | 12.8 ± 1.9 |

Abbreviations: Non-ST elevation myocardial infarction, NSTEMI; Body Mass Index, BMI; Waist Circumference, WC; systolic blood pressure, SBP; diastolic blood pressure, DBP; total cholesterol, TC; triglyceride, TG; high-density lipoprotein cholesterol, HDL—C; low-density lipoprotein cholesterol, LDL-C; glycosylated hemoglobin, HbA1c; homeostatic model assessment for insulin resistance, HOMA-IR; high-sensitivity C reactive protein hs-CRP; erythrocyte sedimentation rate, ESR; gamma-glutamyltransferase, GGT; aspartate transaminase, AST; alanine transaminase, ALT; alkaline phosphatase, ALP; creatine kinase myoglobin, CK-MB; lactate dehydrogenase, LDH; troponin-I, Tn-I.
[a] Progetto cuore http://www.cuore.iss.it/.

background information (including lifestyle and medical history) and blood samples for biochemical measurements and PBMC isolation. In order to calculate the sample size of the study we assumed to detect a difference of at least 2 in the functional properties of HDL and RCT between systemic and intra-coronary arterial microenvironment, a significance level of 0.05 and power of 80% obtaining a total of 20 subjects to enrol.

The study protocol was approved by the Ethical Committee of the Azienda Ospedaliera of Bari, Italy (code 1052). All patients gave their written informed consent for the use of clinical data and blood samples. This study was performed in accordance with the principles of the Declaration of Helsinki.

### 2.2. Biochemical measurements

Biochemical markers of myocardial infarction, glucose and lipid metabolism, liver and renal function, complete blood count, iron status and inflammation were evaluated by standard biochemical methods. The homeostatic model assessment for insulin resistance (HOMA-IR) was determined by the following formula (fasting plasma glucose (FPG) × fasting plasma insulin / 405) [30]. Plasma level of lipoprotein particles containing only apoA-I (LpA-I), particles containing also apoA-II (LpA-I:A-II) was determined by electroimmunodiffusion in agarose gel [31] and preβ-HDL content was assessed by 2-D electrophoresis [32].

### 2.3. Cell culture and assessment of HDL cholesterol efflux

Net cholesterol efflux capacity was measured using Cholesterol Efflux Fluorometric Assay Kit, Cell-Based (Biovision) using a standard, previously validated protocol [33]. Briefly, the murine macrophage cell line J774 (ATCC) was maintained in RPMI 1640 (Life Technologies) supplemented with 8% Fetal Bovine Serum (FBS) (Life Technologies), 100 units/mL penicillin (Sigma), and 100 μg/mL streptomycin (Sigma). At the time of starting the efflux assays, cells were labelled with fluorescent cholesterol according to manufacturer's instructions for 16 h. Post-labeling, cells were gently washed with pure RPMI 1640 and incubated with RPMI supplemented with 2.8% ApoB depleted serum for 5 h. Fluorescence from cell medium and cell lysates were determined using Victor x3 plate reader (PerkinElmer) at Ex/Em = 482/515 nm. Cholesterol efflux was measured as: (fluorescence intensity of the media / total fluorescence intensity of the sum of media and cell lysate) * 100. Cells exposed to serum free medium were used as negative controls in order to determine the final % of efflux derived from serum properties and thus exclude osmotic efflux from cells to cell medium. Each serum was assessed in triplicates and repeated if the standard deviation between wells exceeded 3,0. To correct for inter-assay variations, control samples was included on each plate and used for normalization in subsequent analyses.

### 2.4. Isolation of PBMC and $CD14^+$ cells

Fresh whole blood was collected from systemic arterial and coronary blood in heparinized anti-coagulant tubes (Vacuette®, Greiner Bio-One, Kremsmunster, Austria). PBMCs were isolated using standard validated protocol [34]. PBMCs were employed to isolate $CD14^+$ cells through magnetic labelling (CD14 microbeads, Miltenyi Biotec) according to manufacturer's instructions.

Detailed experimental procedures (PBMC isolation, RNA processing, RTqPCR, RNA sequencing, cytokines assay and statistical analysis) are provided in the Supplementary Material (Appendix A).

## 3. Results

### 3.1. Clinical characterization of the study population

The population of 21 consecutive NSTEMI patients consisted of 14 men and 7 women with a mean age of 63 years enrolled during 12 months of recruitment. Clinical characterization of NSTEMI patients is shown in Table 1. Compared to reference values, NSTEMI patients displayed increased weight, WC, glucose, HbA1c, homocysteine and CRP. As expected, cardiac enzymes (CK-MB, LDH, Tn-I) as well as cardiovascular risk scores (Progetto Cuore) resulted increased.

### 3.2. Lipid profile in systemic arterial and coronary blood of NSTEMI patients

In order to identify the lipid expression profile of NSTEMI patients, we first analysed systemic arterial and coronary blood of these subjects. We observed significant reductions of total cholesterol, LDL, triglycerides and phospholipids levels in coronary blood compared to systemic arterial blood (Fig. 1A–B). Interestingly, no difference was found in HDL-C levels in coronary blood compared to arterial blood of NSTEMI patients (Fig. 1B). Furthermore, we performed univariate linear regression analyses to study coronary, systemic venous and arterial HDL-C levels and we observed a significant linear relationship of HDL-C levels in these compartments (Fig. 1C). These data surprisingly show that in NSTEMI patients in intracoronary compartment the quantity of HDL-C is identical to that the systemic thus *bona fide* reducing the relevance of the measure of HDL-C amount to justify the atherogenic scenario.

### 3.3. HDL subpopulations, apolipoprotein profile and cholesterol efflux capacity (CEC) in systemic arterial and coronary blood of NSTEMI patients

Since it has been demonstrated that HDL-C levels do not represent the anti-atherogenic potential of different HDL subpopulations [35], we decided to study HDL subpopulations in coronary blood. We did not observe any differences in HDL levels between systemic arterial and coronary blood in our population (Fig. 1B). In contrast, in NSTEMI patients we found a significant reduction of the LpAI:LpAII particles, with no changes in LpAI, and a significant reduction in the pre-β HDL fraction in coronary blood compared to systemic arterial blood (Fig. 2A). Together with the apolipoprotein profile, this scenario depicts an intracoronary proatherogenic phenotype and suggests that the systemic profile does not represent a diagnostic and prognostic role in NSTEMI patients.

We then evaluated the capacity of cholesterol efflux (CEC) of serum of NSTEMI patients. In coronary serum the CEC was significantly reduced compared to the systemic serum in agreement with the reduction of the small discoidal pre-beta HDL (Fig. 2B).

Since, apolipoproteins can be considered better predictors of coronary heart disease than lipids, it has been estimated that apo B to apo AI ratio is a good diagnostic measurement of coronary risk [36]. Furthermore, several studies have demonstrated that Apo AI levels can be used as predictor factor of atherosclerosis [37]. In our NSTEMI patients, we observed a significant reduction of Apo AI, Apo AII, Apo B and Apo E levels in coronary blood compared to systemic arterial blood of these patients (Fig. 2C), thus depicting a pro-atherogenic apolipoprotein profile specific in the coronary microenvironment. Overall, while no difference in HDL-C levels has been observed in post-infarction coronary arterial blood of NSTEMI patients, here we show that both the pre-β HDL fraction as well as the apolipoproteins are significantly reduced, thus supporting the result of the reduced coronary serum ability to accept cholesterol.

### 3.4. mRNA sequencing in systemic and coronary PBMC isolated from NSTEMI patients

mRNA sequencing analysis was performed to evaluate genes and pathways differential expressed in systemic and coronary PBMC isolated from NSTEMI patients. We identify 16 significantly down-regulated

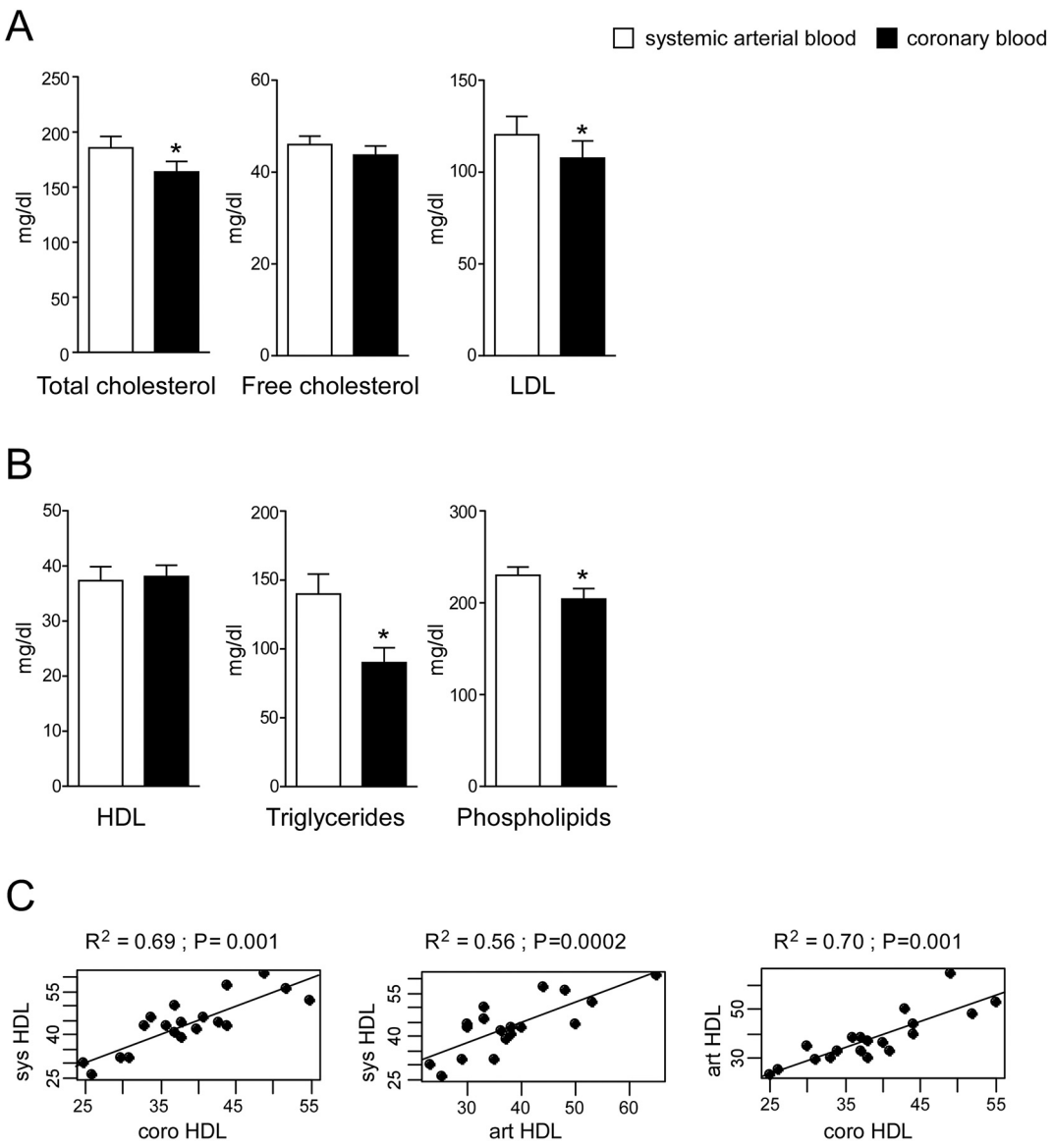


**Fig. 1.** Lipid profile in NSTEMI patients. (A) Total cholesterol, free cholesterol, LDL, (B) HDL, triglycerides and phospholipids levels in serum isolated from arterial and coronary blood of NSTEMI patients. Data are represented as a mean ± standard error (SEM) * = $p \leq 0.05$. (C) Univariate linear regression of coronary (coro), systemic (sys) and arterial (art) HDL levels. The results are indicated as *p*-value of regression coefficient and coefficient of determination ($R^2$).

genes and 12 significantly up-regulated genes in coronary PBMC of NSTEMI patients. In order to explain our data, we used the Core Function of Ingenuity System Pathway Analysis (IPA) to functionally cluster significant biological pathways. We assumed 'biologically relevant' only then 'statistically significant' items comprised in 'significantly modulated' pathways and networks. Intriguingly, the analysis of mRNA expression in coronary monocytes revealed a modulation of networks involved in "infectious diseases, inflammatory disease, inflammatory response", "cardiovascular disease, hematological system development and function, organismal injury and abnormalities" and "cell cycle, gene expression, organismal injury and abnormalities" (Fig. 3A–B). Interestingly, mRNA sequencing revealed a significant reduction in the expression of genes modulated in inflammatory pathways specifically in intracoronary monocytes. Several studies indicate a close relation between inflammation and RCT-related gene expression. In human macrophages, the reduction of pro-inflammatory stimuli promotes cholesterol efflux through ABCA1 [38]. LXR is able to reduce inflammation through changes in the cholesterol metabolism *via* ABCA1 activation. Macrophages isolated from ABCA1-deficient mice presented high levels of chemokines, growth factors and cytokines [39]. In line with this, we therefore analysed the serum levels of inflammatory cytokines MCP-1, IFN-γ and CCL4 (Fig. 4A) thus underscoring a specific immediate post-infarction anti-inflammatory action of intracoronary monocytes.

### 3.5. *Cholesterol efflux-related gene expression from monocytes (CD14+ cells) isolated from systemic and coronary microenvironment of NSTEMI patients*

We then studied the expression of genes related to cholesterol efflux in monocytes (CD14+ cells) isolated from systemic arterial and coronary blood of NSTEMI patients. Monocytes and mature macrophages play a pivotal role in myocardial infarction and they can contribute differently to each stage of the disease rapidly adapting their phenotype/function [40]. In the coronary microenvironment of NSTEMI patients, we found a significant up-regulation of LXRα, ABCA1 and APOE gene expression levels compared to systemic compartment (Fig. 4B). These data may indicate that immediately after myocardial infarction macrophages activate the LXR driven transcriptional machinery to expel cholesterol and to promote anti-inflammatory action [41].

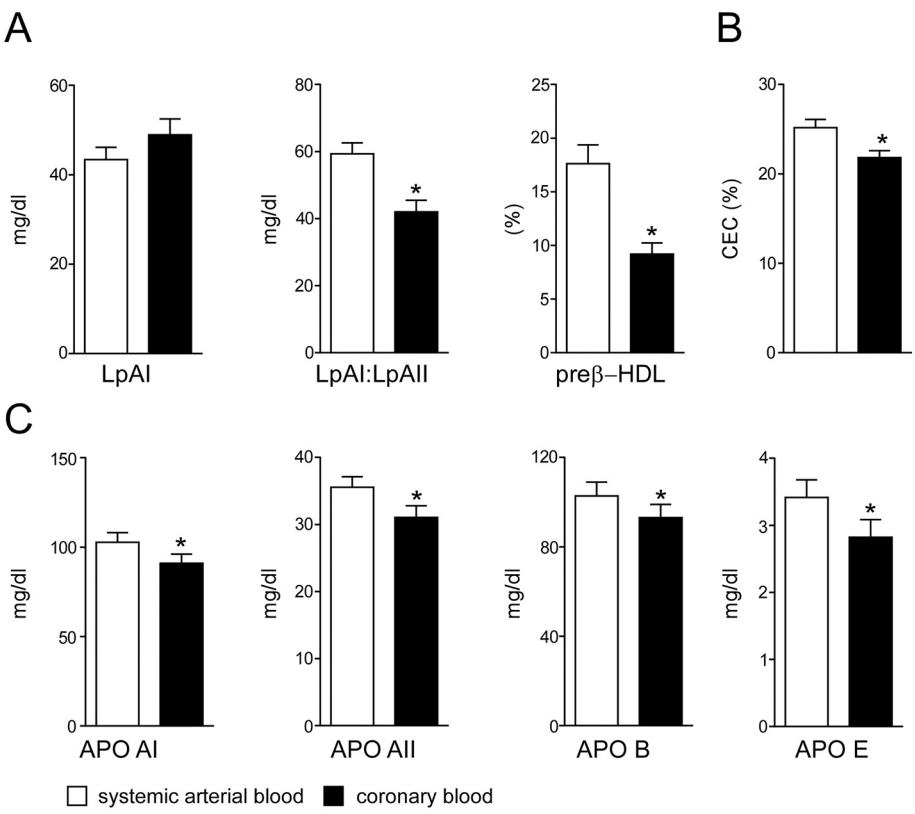


**Fig. 2.** HDL subpopulations, apolipoprotein profile and cholesterol efflux capacity (CEC) in systemic arterial and coronary blood of NSTEMI patients. (A) HDL subpopulations (LpAI, LpA/AII and pre-β HDL) profile in systemic arterial and coronary blood of NSTEMI patients is reported. The results are expressed as mean ± SEM. Statistical significance ($P < 0.05$) was assessed by Student's *t*-test. (B) Cholesterol efflux capacity (CEC) in systemic arterial and coronary serum of NSTEMI patients assessing through fluorimetric assay. The results are expressed as mean ± SEM. Statistical significance ($P < 0.05$) was assessed by Student's t-test. (C) ApoAI, ApoAII, ApoB and ApoE levels in serum isolated from systemic arterial and coronary blood of NSTEMI patients. Data are represented as a mean ± SEM, * = $p \leq 0.05$.

## 4. Discussion

The present study focused on the relevance of RCT in systemic and coronary arterial blood. In our study, we evaluated the changes in coronary and systemic microenvironment of NSTEMI patients. In particular, in these two compartments we analysed the serum ability to accept cholesterol and the cholesterol efflux capacity of monocytes/macrophages. We demonstrate that in coronary microenvironment of NSTEMI patients these important atheroprotective mechanisms are impaired and uncoupled. Indeed, while the HDL-C quantity was similar between systemic and coronary arterial blood, in NSTEMI patients we observed a significant reduction of preβ-HDL in the coronary *versus* systemic microenvironment fraction measured in coronary *versus* systemic serum. On the contrary, compared to systemic arterial monocytes, in coronary microenvironment of NSTEMI patients the monocytes exhibited a higher mRNA expression of nuclear receptor LXRα and its targets ABCA1 and APOE which drive cholesterol efflux capacity. NSTEMI is an acute coronary syndrome, with coronary arteries obstruction and consequent reduction of blood flow to the heart and myocardial infarction. The etiology of NSTEMI is represented by atherosclerosis that is the result of the involvement of multiple factors, such as impaired circulating lipoprotein levels, hemodynamic factors, endothelial and smooth muscle cells, macrophages, T lymphocytes, hypertension, dyslipidemia and central obesity. In the present study, we focused on intracoronary HDL functionality. Elevated HDL-C levels are associated with low cardiovascular disease risk [3–5]. Recently, it has been demonstrated that acute myocardial infarction is characterized by HDL dysfunctionalities despite similar HDL cholesterol levels [42], while genetically-determined subjects with low HDL-C levels might show total absence of myocardial infarction [22]. These data support the hypothesis that HDL function is a better indicator for cardiovascular risk than plasma HDL-C concentration [43,44]. Nothing is known about the functionality of HDL in the coronary microenvironment, where the damage takes place, compared to peripheral circulatory system. Thus, here we depicted the systemic and coronary microenvironment of NSTEMI patients in order to evaluate serum ability to accept cholesterol and monocytes/macrophages cholesterol efflux.

Khera *et al.* have demonstrated an inverse relationship between the ability of HDL to promote cholesterol efflux from macrophages and CAD, independently of the HDL levels [24]. The serum ability to accept cellular cholesterol is influenced by HDL structure that behaves as cholesterol acceptor. Preβ-HDL particles are correlated with high ABCA1-mediated efflux [17,18] and they denote the serum ability to accept cholesterol from the environment. Therefore, we analysed HDL-C concentration, the apolipoprotein profile, and preβ-HDL levels in systemic arterial and coronary blood of NSTEMI patients showing in the coronary microenvironment a significant reduction of pre-β HDL fraction compared to systemic compartment. This result is confirmed by a significant CEC reduction in the coronary microenvironment compared to the systemic serum of NSTEMI patients. CEC shows the HDL ability to induce cholesterol efflux from macrophages and it is considered an index of HDL composition and function [20]. Moreover, it has been demonstrated that CEC is a marker of intima-media thickness better than HDL levels [24] and it is inversely correlated with coronary artery disease [45].

Together with serum ability to accept cholesterol, the initial step in RCT is macrophages capacity to expel cholesterol. Indeed, macrophage cholesterol efflux is correlated with atherosclerosis and represents a new marker of cardiovascular risk [46]. The YELLOW II (Reduction in Coronary Yellow Plaque, Lipids and Vascular inflammation by Aggressive Lipid Lowering) study analysed the influence of statin therapy (rosuvastatin) on plaque morphology, cholesterol efflux and PBMC gene profiling in patients with stable CAD underwent percutaneous coronary intervention for a culprit lesion. In these patients, statin therapy modulated plaque stabilization enhancing cholesterol efflux and promoting PBMC transcriptomic changes in inflammation, cholesterol synthesis, uptake and efflux [47].

The efflux process involves several players such as ApoE and ABCA1. ApoE is able to induce cholesterol efflux also in the absence of cholesterol acceptors leading to the production of nascent HDL [28]. The ABC

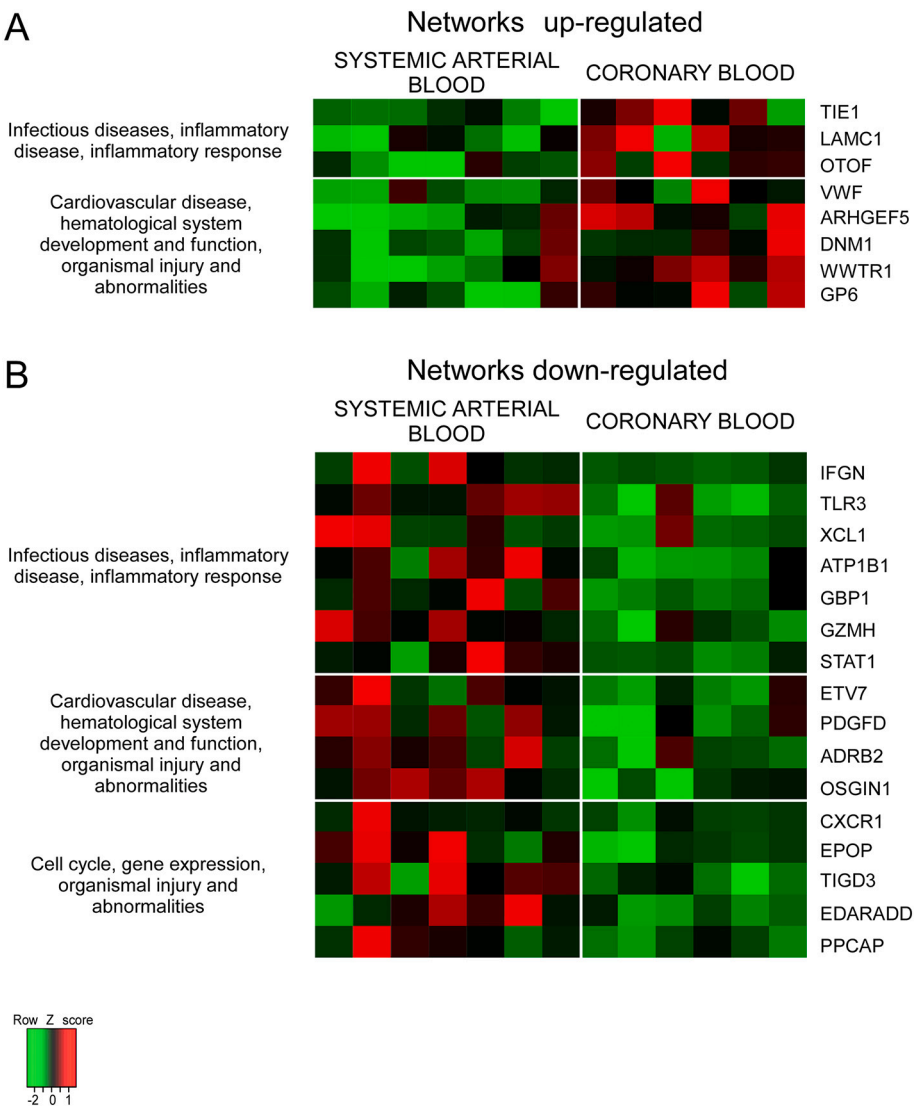


**Fig. 3.** mRNA sequencing in PBMC isolated from systemic and coronary microenvironment of NSTEMI patients. (A) Genes up-regulated and (B) down-regulated in PBMC isolated from systemic and coronary microenvironment of NSTEMI patients using mRNA sequencing, and clustered in networks displaying a coordinate biological function. Data are shown in a heatmap with a matrix format of the genes differentially modulated within the specific network; single rows represent gene expression in a single patient (column). Colors: red, expression greater than the mean; black, expression equal to the mean; green, expression smaller than the mean. Lateral bars: fold changes among groups.

transporter, ABCA1, enhances the trafficking of cholesterol and phospholipids [48] and its gene expression is induced by the nuclear receptor LXR, the transcriptional regulator of lipid homeostasis [25]. In human macrophages, the reduction of pro-inflammatory *stimuli* promotes cholesterol efflux through ABCA1 [38]. Furthermore, Ito *et al.* demonstrated the ability of LXR to reduce inflammation through changes in the cholesterol metabolism. The nuclear receptor LXR activates the transcription of ABCA1 modifying membrane cholesterol homeostasis and leading to secondary effect on inflammation *via* the inhibition of NF-kB and MAPK signaling pathways [49].

The administration of a synthetic LXR agonist increases RCT of macrophage-derived cholesterol *in vivo* [50]. Lo Sasso *et al.* showed that intestinal LXR activation induced the expression of LXR target genes involved in cholesterol secretion decreasing cholesterol absorption and increasing pre-βHDL levels [51]. Activation of LXR in coronary macrophages could be important to regulate macrophages capacity to expel cholesterol.

Oxysterols, the metabolites of cholesterol, have been identified as potent LXR agonists. Umetani *et al.* have demonstrated that 27-hydroxycholesterol concentration, an endogenous LXR ligand, is elevated in foam cells and atherosclerotic plaques [52], and thus could eventually play a role in the activation of LXR in coronary macrophages of NSTEMI patients.

LXR is involved in the transcriptional regulation of macrophage activation and exerts transcriptional control over an anti-inflammatory macrophage pathway [41]. In our study, in coronary microenvironment of NSTEMI patients, we observed in CD14+ cells (mostly monocytes) a significant up-regulation of LXRα, ABCA1 and APOE demonstrating that coronary monocytes are estimated to secrete more cholesterol compared to systemic monocytes. In line with mRNA sequencing data, we observed a reduction of the inflammatory molecules MCP-1, IFN-γ and CCL4 confirming the link between LXR, ABCA1 activation and the reduction of inflammatory status. The coronary monocytes ability to secrete cholesterol could represent an adaptation mechanism after myocardial infarction. Thus, in the immediate post infarction period, there is a putative activation of the LXR driven cholesterol efflux capacity of intracoronary monocytes to overcome the reduction of serum ability to accept cholesterol due to lower preβ-HDL fraction in the coronary microenvironment. This mechanism could represent an option to restore the serum ability to accept cholesterol leading to a *bona fide* better prognosis of myocardial infarction.

## CRediT authorship contribution statement

**Marica Cariello:** Conceptualization, Methodology, Investigation, Writing – original draft. **Roberto Salvia:** Investigation. **Jennifer Härdfeldt:** Investigation. **Marilidia Piglionica:** Investigation. **David Rutigliano:** Resources. **Pasquale Caldarola:** Resources. **Alice Ossoli:**

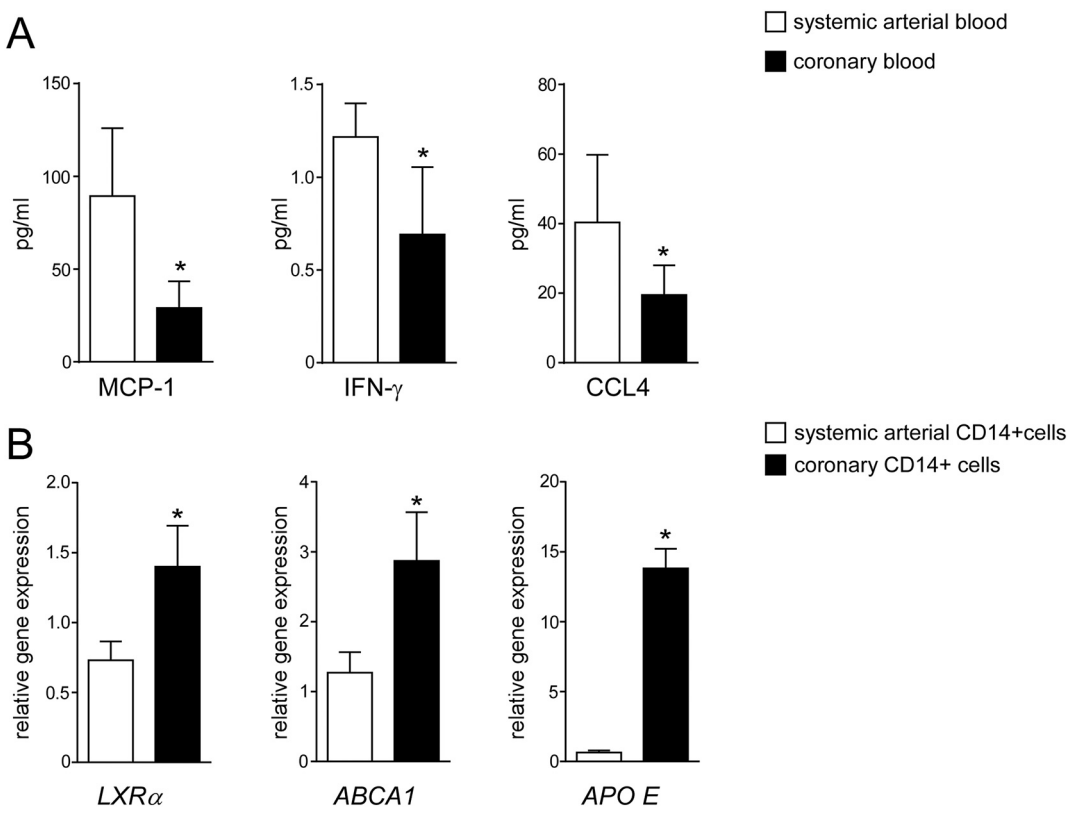


**Fig. 4.** Cholesterol efflux from monocytes (CD14+ cells) isolated from systemic and coronary microenvironment of NSTEMI patients. (A) Serum inflammatory cytokines levels in systemic and coronary microenvironment of NSTEMI patients assessing through Bioplex analysis. The results are expressed as mean ± SEM. Statistical significance ($P < 0.05$) was assessed by Student's t-test. (B) Gene expression analyses of LXRα, ABCA1 and APOE in CD14+ cells isolated from systemic and coronary blood of NSTEMI patients. Cyclophilin was used as a housekeeping gene to normalize data. The results are expressed as mean ± SEM. Statistical significance ($P < 0.05$) was assessed by Student's t-test.

Investigation. **Michele Vacca:** Data curation, Formal analysis. **Giusi Graziano:** Data curation, Formal analysis. **Stefano Battaglia:** Data curation, Formal analysis. **Roberta Zerlotin:** Investigation. **Maria Arconzo:** Investigation. **Lucilla Crudele:** Investigation. **Carlo Sabbà:** Supervision. **Laura Calabresi:** Supervision. **Antonio Moschetta:** Writing – review & editing, Project administration, Funding acquisition.

## 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

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