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Low-grade lipopolysaccharide-related endotoxemia alters coronary thrombus composition and fibrin clot properties in patients with acute ST-segment elevation myocardial infarction

Short title: LPS affects coronary thrombus composition and fibrin clot properties

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INTRODUCTION

ST-segment elevation myocardial infarction (STEMI) frequently results from acute coronary flow cessation due to atherosclerotic plaque erosion or disruption followed by intracoronary thrombus formation (ICT) [1]. ICT composition and structure assessed using scanning electron microscopy (SEM) indicates a dominance of fibrin (60%) if thrombi are retrieved by thrombectomy within the first 12 hours since symptom onset [2]. In STEMI patients the contribution of components within thrombi varies with time, which may affect their stability and resolution [2]. Cardiovascular risk factors, together with low-grade inflammation, promote prothrombotic fibrin clot properties, i.e., faster polymerization and formation of denser fibrin networks composed of thinner fibers, leading to increased resistance to fibrinolysis [3].

Alterations in the intestinal microbiome characterized by the reduction of commensal in favor of pathogenic bacteria, i.e., a gut dysbiosis, has been recently demonstrated as a proinflammatory and prothrombotic factor acting via translocation of bacterial products into the systemic circulation [4, 5]. Lipopolysaccharide (LPS), a glycolipid component of Gram-negative bacteria outer membrane, is detectable in blood, and increased intestinal permeability along with impaired LPS degradation rise circulating LPS concentrations. LPS has been found to act mainly via toll-like receptor 4 expressed among others in immune and endothelial cells [6].

Carnevale et al. [4] reported LPS-positive areas within ICT, along with increased plasma P-selectin, a marker of platelet activation, associated with low-grade endotoxemia and gut permeability. They also found positive correlations of serum LPS with white blood cell (WBC) count and C-reactive protein [4]. Previously, we reported positive correlations of fibrinogen, P-selectin, plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor antigen, soluble CD40 ligand, and myeloperoxidase with thrombus fibrin content [7]. After adjustment for fibrinogen and onset-to-thrombectomy time circulating von Willebrand factor antigen, myeloperoxidase, and P-selectin together with renal impairment, arterial hypertension, and onset-to-thrombectomy time were the independent predictors of fibrin-rich ICT formation [7]. It has been demonstrated that formation of denser plasma fibrin clots was independently associated with high fibrin content within the ICT [8]. Little is known about links between coronary thrombus composition and LPS-mediated inflammation [4, 9]. We hypothesized that prothrombotic plasma fibrin clot properties and the high thrombus fibrin content are associated with elevated serum LPS in STEMI patients.

METHODS

The study is a subanalysis of our previous report [7] on ICTs obtained 4.0–16.5 h since chest pain onset by manual aspiration during primary coronary intervention in STEMI patients recruited between January and December 2012. Patients with estimated glomerular filtration rate below 30 ml/min, acute infection, treatment with systemic corticosteroids or oral anticoagulants, malignancy, and in-stent thrombosis were excluded. SEM details and laboratory tests used are provided in the Supplementary Materials. The study has been approved by Ethics Committee (6/2011). All participants gave written informed consent.

Statistical analysis

Continuous variables are expressed as mean (standard deviation) or median (interquartile range [IQR]). To reflect the wide ranges of the thrombus components, the exact ranges were provided. Differences were tested by Student t test or by Mann–Whitney U test. Categorical variables were compared by chi-square or Fisher exact test. The Pearson or Spearman rank correlation coefficients were calculated. A *P*-value <0.05 was considered statistically significant. Statistical analyses were performed with IBM SPSS Statistics 21.0.

RESULTS AND DISCUSSION

The study patients have been presented elsewhere [7]. Forty patients (24 [60%] women) aged 36 to 90 years were studied (16 patients in the pre-percutaneous coronary intervention (PCI) group and 24 patients in the post-PCI group). Cardiovascular risk factors distribution and past medical history were similar in both groups (Supplementary material, *Table S1*). Median symptom onset-to-thrombectomy time was 390 min (IQR 240–990 min) in the pre-PCI group and 420 min (IQR 285–885 min) in the post-PCI group. Median LPS in the pre-PCI group was 36.1 (34.9–38.9) pg/ml (range 32.8–44.9 pg/ml), and 35.9 (33.9–39.0) pg/ml (range 32.4–41.2 pg/ml) in the post-PCI group. There was no association of LPS with age, sex, comorbidities, and medications used in either group. In the post-PCI group LPS was positively correlated with the onset-to-thrombectomy time (r = 0.54; *P* = 0.002). The pre-PCI group with LPS above median had greater cardiac troponin T on admission (Supplementary material, *Table S1*) and LPS was correlated with WBC count (r = 0.5; *P* = 0.001). Platelet count, fibrinogen, or markers of platelet, neutrophil, and endothelial activation showed no associations with LPS (Supplementary material, *Table S1*).

Analysis of 323 SEM images showed that a mean fibrin, red blood cell (RBC), platelet and white blood cell content were estimated at 49.1% (range 2.7%–88.5%), 24.2% (range 0%–58.7%), 11.6% (range 0.1%–65.8%), and 3.7% (range 0%–31.3%), respectively. In the post-PCI group with LPS levels >35.9 pg/ml, thrombus RBC content was lower, while fibrin content was greater compared to the remainder (25.8% [19.7–46.6] vs. 45.5% [27.9–63.0]; *P* = 0.04), and this association was even stronger in patients with LPS levels in the top quartile compared to the reminder (28.3% [19.3–48.8] vs. 59.6% [32.7–77.1]; *P* = 0.024). In the post-PCI group serum LPS was positively correlated with fibrin content (r = 0.52; *P* = 0.007) and negatively with RBC content (r = –0.47; *P* = 0.018). In the pre-PCI groups these correlations were absent (Figure 1).

In the pre-PCI group, patients with LPS levels >36.1 pg/ml had longer clot lysis time (CLT) (Supplementary material, *Table S1*). LPS was negatively associated with K_s-TF (r = –

0.47; *P* = 0.017) in the pre-PCI group, while positive correlations of LPS with CLT were observed in pre- and post-PCI groups (r = 0.74; *P* = 0.012, and r = 0.36; *P* = 0.045, respectively, Figure 1). No associations with fibrinolysis-related proteins, including PAI-1, a major regulator of CLT, were detected.

Our study is the first to show that greater ICT fibrin content, along with lower plasma clot permeability and reduced lysability are associated with increased serum LPS levels in STEMI patients. This suggests that endotoxemia in STEMI may affect fibrin clot phenotype and thrombus composition. In the current study thrombus fibrin- and RBC content has been found to have a new modulator, i.e., LPS, which correlated with WBC and cardiac troponin T on admission, but not with other markers of inflammation and myocardial or endothelial injury.

Nunes et al. [10] investigated LPS-dependent clot formation and architecture, but they did not study clot properties. Ząbczyk et al. [11] reported that endotoxemia may contribute to increased thrombin generation and PAI-1-mediated hypofibrinolysis in patients with pulmonary embolism [11]. This is consistent with our findings on the LPS-mediated antifibrinolytic effect of LPS in patients with atrial fibrillation [12]. Together with CLT we also measured K_s, a key measure reflecting a pore size within fibrin networks. Lower K_s and prolonged CLT indicate a prothrombotic clot phenotype. In our study K_s was negatively correlated with CLT in the pre- and post-PCI groups (r = –0.7; *P* = 0.006 and r = –0.47; *P* = 0.012, respectively), which reflects the data consistency. An inverse association of K_s with LPS in the pre-PCI group suggests that low grade endotoxemia in STEMI contributes to dense fibrin clot formation, likely via inflammatory mechanisms.

Study limitations should be acknowledged. The sample size is small and the patients were assessed pre- and post-PCI in terms of laboratory tests, therefore our results are preliminary, and did not refer to the present antiplatelet strategies [13] with infrequent use of thrombectomy in STEMI. Zonulin, a marker of gut permeability, was not assessed by us, however it is well established that the main source of circulating LPS in MI patients is the gut [4, 14] with unlikely contribution of other sources. However, we believe that the impact of elevated LPS on ICT composition may be evaluated regardless of its origin and that the hypothesis-generating results may stimulate further investigation in the field of endotoxemia-related hemostatic alterations.

We conclude that STEMI patients with low-grade LPS-mediated endotoxemia tend to form fibrin-rich intracoronary thrombi in association with less permeable and resistant-to-lysis plasma clots.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

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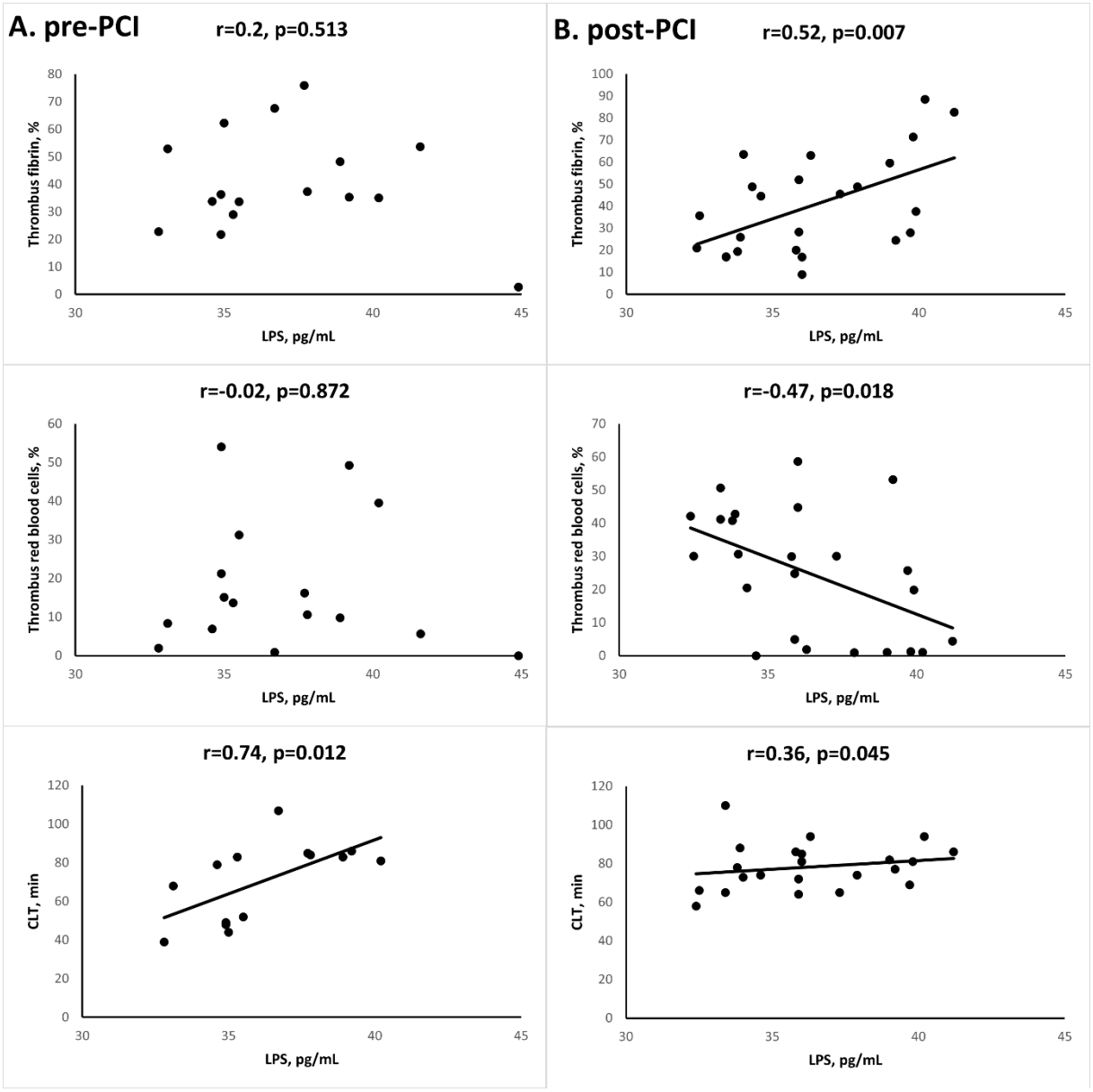

Figure 1. Correlations between serum lipopolysaccharide (LPS) levels and intracoronary thrombus contents, and serum LPS levels and clot lysis time (CLT) in the pre-percutaneous coronary intervention (PCI) (**A**) and post-PCI (**B**) groups