Clinical Research in Cardiology (2019) 108:950–962
https://doi.org/10.1007/s00392-019-01425-x

ORIGINAL PAPER

Polyhedral erythrocytes in intracoronary thrombus and their association with reperfusion in myocardial infarction

Jaroslaw Zalewski[1] · Lukasz Lewicki[2,3] · Krzysztof Krawczyk[1] · Michal Zabczyk[4] · Radoslaw Targonski[3] · Patrycja Molek[1] · Jadwiga Nessler[1] · Anetta Undas[4]

Received: 4 November 2018 / Accepted: 29 January 2019 / Published online: 1 February 2019

Abstract

Objective The tightly packed arrays of polyhedral erythrocytes, polyhedrocytes, formed during thrombus contraction, have been detected in some intracoronary thrombi (ICT) obtained from patients with ST-segment elevation myocardial infarction (STEMI). We sought to investigate determinants of polyhedrocyte content in ICT and its association with reperfusion in STEMI.

Methods We assessed the composition of ICT obtained during thrombectomy within 12 h since the symptom onset in 110 STEMI patients, following 300 mg of aspirin ($n=110$) and 600 mg of clopidogrel ($n=75$). The predominance of fibrin, erythrocytes, polyhedrocytes or platelets was evaluated using scanning electron microscopy.

Results Polyhedrocytes were found in 34 (30.9%) ICT, in which they covered 20–50% (median 38.8%) fields of view. Patients with polyhedrocytes in ICT had lower median minimal reference infarct-related artery (IRA) diameter by 20% ($p<0.0001$) and area by 31% ($p<0.0001$) versus those without polyhedrocytes. Time of ischemia showed association with the polyhedrocyte content ($r=0.26$, $p=0.007$). By multivariate analysis, minimal IRA diameter ($\beta=-0.50$, $p<0.0001$) and ischemia time ($\beta=0.20$, $p=0.035$) independently affected polyhedrocyte content in ICT ($R^2=0.45$, $p<0.0001$). Patients with ischemia time of > 3 h and polyhedrocytes present in ICT had more frequently TIMI-2/3 flow after thrombus aspiration (96% vs. 67%, $p=0.02$) and final TIMI-2/3 myocardial perfusion grade (92% vs. 57%, $p=0.044$) versus those without polyhedrocytes.

Conclusions Our findings indicate that the presence of polyhedrocytes in ICT, observed in one-third of STEMI patients, is associated with smaller minimal IRA diameter, prolonged ischemia and their formation in late presenters is associated with more effective thrombus aspiration and better myocardial reperfusion.

Keywords Polyhedrocytes · Thrombus · Myocardial infarction · Scanning electron microscopy

Introduction

Reduced in-hospital and long-term mortality in patients with acute myocardial infarction (MI) within the last decade is associated with improved pharmacological and invasive treatment [1–3]. Despite high rates of implementation of guideline recommended antithrombotic therapies including aspirin, clopidogrel, more potent $P2Y_{12}$ inhibitors, heparins and bivalirudin [4], thrombus-related complications during mechanical reperfusion such as distal embolization, residual thrombus [5, 6], acute stent thrombosis [7] and especially the no-reflow phenomenon are still common [8–10] and their occurrence is usually associated with worse clinical outcomes [11, 12].

Plaque rupture associated with the exposure of collagen and tissue factor triggers both platelet activation, thrombin

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00392-019-01425-x) contains supplementary material, which is available to authorized users.

✉ Jaroslaw Zalewski
jzalews@szpitaljp2.krakow.pl

1 Department of Coronary Artery Disease, Jagiellonian University Medical College, 80 Pradnicka Street, 31-202 Krakow, Poland

2 Gdansk University of Technology, 11/12 Narutowicza Street, 80-233 Gdansk, Poland

3 Pomeranian Cardiology Centers, 10 Jagalskiego Street, 84-200 Wejherowo, Poland

4 Department of Experimental Cardiac Surgery, Anesthesiology and Cardiology, Jagiellonian University Medical College, 80 Pradnicka Street, 31-202 Krakow, Poland

generation and then the formation of fibrin, a major component of intracoronary thrombus (ICT) [13, 14]. Fibrin–platelet meshwork of ICT is then contracted with simultaneous entrapment of large amounts of erythrocytes and inflammatory cells. Recently, uniquely shaped compressed polyhedral erythrocytes, called polyhedrocytes, have been found using scanning electron microscopy in the whole blood clots formed in vitro [15]. Confocal light microscopy with labeled erythrocytes, platelets and fibrin(ogen) allowed to ensure that those specific structures were not an artifact and appeared as a result of clot contraction. In vitro clot formation was associated with fibrin exposure on clot surface and erythrocyte compression in the interior core into the tightly packed arrays of polyhedrocytes [15]. In vitro studies showed a stepwise clot contraction starting from its initiation followed by linear contraction and clot stabilization [16]. Moreover, thrombin, calcium ions, cross-linking by active factor XIII, and platelets promoted clot contraction, while red blood cells impaired contraction and reduced clot elasticity [16]. In turn, polyhedrocytes have been observed in vivo in human arterial and venous thrombi as well as pulmonary emboli [17–19]. Moreover, it was found that the kinetics and extent of clot contraction can be different also in patients with acute ischemic stroke [20].

Little is known about factors that determine polyhedrocyte formation during ICT contraction in vivo. Fast clot contraction with a large content of polyhedrocytes in acute MI may facilitate myocardial perfusion restoration during mechanical recanalization of the infarct-related artery (IRA). Based on the previous studies [16, 18], we hypothesized that polyhedrocyte formation in the contracting ICT among MI patients is modulated by systemic factors and local environment. Therefore, we sought to investigate determinants and clinical significance of the polyhedrocyte content in ICT during STEMI.

Patients and methods

We studied consecutive STEMI patients treated in two centers within the first 6 months of 2016 with primary percutaneous coronary intervention (PCI) preceded by aspiration thrombectomy. The inclusion criteria were chest pain onset within 12 h with concomitant ST-segment elevation of ≥ 1 mm in at least two contiguous leads or ≥ 2 mm in at least two contiguous precordial leads. The exclusion criteria were cardiogenic shock on admission, previous MI at the same territory, previous coronary artery bypass surgery, history of malignancy, venous thromboembolism or stroke, liver injury (alanine aminotransferase > 1.5 ULN), serum creatinine > 177 µmol/L and current oral anticoagulation.

At first medical contact all patients received 300 mg of aspirin, 72 (65.5%) of them were loaded with 600 mg of clopidogrel and 75 (68.2%) received intravenous bolus of 5000 IU of unfractionated heparin (UFH). On admission, hemoglobin, hematocrit, red blood cell count, white blood cell count, platelet count, lipid profile, glucose, creatinine, fibrinogen, and high-sensitivity C-reactive protein (CRP) were determined by routine laboratory techniques. Immediately before PCI patients received weight-adjusted bolus of UFH to achieve activated clotting time of 200–250 s. Of 255 eligible consecutive STEMI patients, thrombotic material was successfully removed in 110 (43%) from the IRA mostly with the Export® Aspiration Catheter (Medtronic Inc., Minneapolis, Minnesota, USA), and those patients were included in further analysis.

The study protocol complied with the Declaration of Helsinki was approved by the Ethics Committee of the Jagiellonian University. All patients gave written informed consent.

Thrombus assessment

The ICT preparation and analysis were described previously [18, 21, 22]. Briefly, immediately after aspiration and visual assessment fresh thrombus was fixed with 2.5% glutaraldehyde, rinsed several times in phosphate-buffered saline, dehydrated in ethyl alcohol and then placed into tert-Butyl alcohol. After drying procedure, the outer portion of the thrombi was partly removed to obtain samples of the interior portions of the ICT and all thrombi were coated with gold using JEOL JFC-1300 Ion Sputter (JEOL Ltd., Tokyo, Japan) (Fig. 1a–c i–iii). Next, ICTs were photographed by means of the scanning electron microscope JCM 6000 and JSM 5410 (both JEOL) equipped with an energy dispersion spectrometer for chemical analysis (magnifications in Fig. 1a–c i, ii − 30×, iii − 100×, iv–vi − 3500×). All fields of view (FoV) with magnification of 3500× were randomly selected from different parts of every ICT, covered by a grid 20 × 20 and analyzed using image software [18, 22]. In each of 400 squares of the grid superimposed on the scanning electron microscopic images, the dominant component of ICT was recorded, i.e., (1) fibrin, (2) erythrocytes, or (3) platelets. The content of each ICT component in every FoV was calculated by dividing the number of squares with the same component by 400 and expressed as a percentage. Because the distribution of content of fibrin, erythrocytes and platelets in FoV for every ICT was non-normal, median values were used for further analyses.

The erythrocytes were specified as either normal biconcave disc erythrocytes (Fig. 1a iv–vi, b iv) or polyhedrocytes (Fig. 1c iv–vi, b v). Intermediate forms of erythrocytes (Fig. 1b vi) were included into polyhedrocyte population during analysis. The polyhedrocyte content in ICT was expressed qualitatively as either (1) polyhedrocyte presence in at least one FoV per ICT (named further as polyhedrocyte

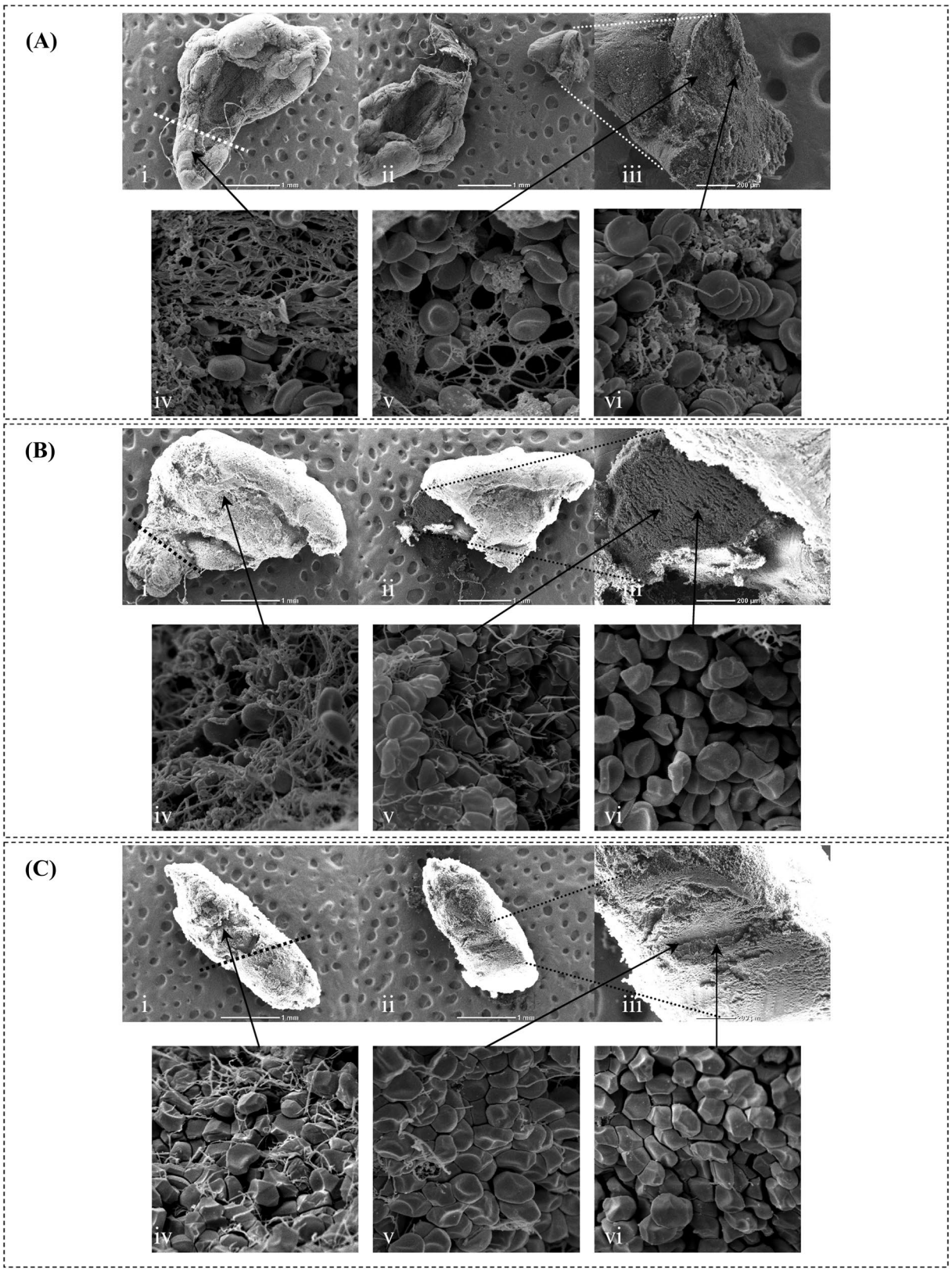

Fig. 1 The intracoronary thrombi (ICT) in scanning electron microscopy. Three intracoronary thrombi (**a**–**c** i with magnification ×30) and representative fields of view with magnification ×3500 from scanning electron microscopy (**a**–**c** iv–vi) from random parts of ICT (**a**–**c** i, iii). **a** ICT with normal, biconcave disc erythrocytes, **b** ICT with polyhedrocytes and intermediate forms, **c** polyhedrocyte-rich ICT. (i) The whole ICT, (ii) after exposure of interior portion, iii. Inner part of ICT with magnification ×100

presence in ICT, Fig. 1b) or as (2) the polyhedrocyte presence in more than 50% of FoV per thrombus (named as ICT rich in polyhedrocyte, Fig. 1c). The polyhedrocyte content in ICT was also expressed semi-quantitatively as a ratio of the number of FoV with present polyhedrocytes to the number of all examined FoV in each ICT. Two independent investigators unaware of any clinical and laboratory data evaluated all thrombi, with the inter-observer and intra-observer agreement of 94% and 96%, respectively.

Angiography analysis

The angiograms were analyzed off-line for the determination of the IRA, epicardial blood flow and distal embolization, based on visual inspection. Reference lumen diameter (RLD) of IRA was measured immediately before the place of culprit lesion with the commercially available software in two contralateral projections (RLD1, RLD2). The lower value of RLD was specified as the minimal RLD (minRLD). Mean value of RLD (mRLD) was calculated as $½ \times (RLD1 + RLD2)$ (mm). In turn, IRA reference lumen area (RLA) was calculated as $¼ \times \pi \times RLD1 \times RLD2$ (mm^2). The epicardial blood flow was evaluated by means of the thrombolysis in myocardial infarction (TIMI) flow scale and myocardial perfusion was scored according to the TIMI myocardial perfusion grades (TMPG) before PCI, after aspiration and after PCI.

Fibrin clot properties

On admission blood samples were drawn atraumatically from an antecubital vein and centrifuged within 30 min at 2500*g* and room temperature.

Clot permeability

Permeation properties of fibrin clots were investigated as described [18, 23]. Briefly, 20 mmol/l calcium chloride and 1 U/ml human thrombin (Sigma-Aldrich, St Louis, MO, USA) was added to citrated plasma. After incubation at room temperature, tubes containing the clots were connected to a reservoir of a buffer (0.05 mol/l Tris–HCl, 0.15 mol/l NaCl, pH 7.5) and its volume flowing through the gels was measured. A permeation coefficient (Ks, 10^{-9} cm^2), which indicates the pore size, was calculated from the equation: $Ks = Q \times L \times \mu / t \times A \times \Delta p$, where Q is the flow rate in time t, L is the length of a fibrin gel, μ is the viscosity of liquid (in poise), A is the cross-sectional area (in cm^2), and Δp is a differential pressure (in dyne/cm^2). The intra-assay variability of results was 7.2%.

Plasma clot lysis assay

Fibrinolysis induced by recombinant tissue plasminogen activator (rt-PA, Boerhinger Ingelheim, Ingelheim, Germany) was evaluated as described [18, 23]. Briefly, 100 µl of citrated plasma was diluted with 100 µl of a Tris-buffer, containing 20 mmol/l calcium chloride, 1 U/ml human thrombin and 1 µmol/l rt-PA. Assembly kinetics was monitored by absorbance at 405 nm in duplicate. Lysis time (t50%, min) was defined as the time required for a 50% decrease in fibrin absorbance. The inter- and intra-assay coefficients of variation were < 7%.

Reperfusion and clinical outcomes

Serum creatine kinase (CK, IU/l), isoenzyme MB of creatine kinase (CK-MB, IU/l) and troponin I (TropI, ng/ml) was measured on admission and every 8 h during the first 48 h after PCI. Maximum of CK-MB ($CK\text{-}MB_{MAX}$) and troponin I ($TropI_{MAX}$) were analyzed.

Before discharge left ventricular ejection fraction was evaluated by two-dimensional echocardiography. A 30-day clinical outcome included death, recurrent MI and recurrent hospitalization due to symptoms of heart failure.

Statistical analysis

In this study, there was no formal sample size calculation because of lack of primary endpoint. However, based on previous polyhedrocyte prevalence in ICT of about 20% [18], the study was powered to have a 90% chance of detecting a correlation between polyhedrocyte content and minimal IRA lumen diameter of more than $|-0.33|$ using a p value of 0.05. To demonstrate such an association or greater, 100 patients were required in the study group.

Statistical analyses were performed with SPSS 20.0 software. Continuous variables are expressed as mean ± SD or median (interquartile range) and categorical variables as number (percentage). Continuous variables were first checked for normal distribution by the Shapiro–Wilk statistic and compared by Student t test when normally distributed or by the Mann–Whitney U test for non-normally distributed variables. Categorical variables were analyzed by Chi-square or Fisher's exact test. The Pearson or Spearman rank correlation coefficients were calculated to test the association between two variables with a normal or non-normal distribution, respectively. All clinical, biochemical and coronary angiography parameters associated ($p < 0.2$) with the content of polyhedrocytes in an univariate model and not correlated with another independent variable were then included in the multivariate regression analysis to determine predictors of polyhedrocyte content in ICT. A two-sided $p < 0.05$ was considered statistically significant.

Results

Polyhedrocytes in ICT

Patients baseline and procedural characteristics are shown in Tables 1 and 2. A total of 1061 fields of view within 110 thrombi were assessed, with a median (IQR, interquartile range) of nine fields (8–10) per thrombus. Polyhedrocytes were found in 34 (30.9%) ICT, in which they were observed in 20–50% (median 38.8%) of fields of view. Moreover, ICT rich in polyhedrocytes were found in 11 (10%) patients. In all ICT, the median fibrin content was 55.7 (35.5–70.9)%, erythrocyte content was 29.1 (15.6–48.9)% and platelet content was 3.1 (0.9–11.7)%.

Table 1 Baseline characteristics

	ICT without polyhedrocytes, $n=76$	ICT with polyhedrocytes, $n=34$	p value
Age, (years)	63.1 ± 12.4	64.1 ± 11.2	0.67
Male gender	50 (65.8)	23 (67.6)	0.85
Body mass index, (kg/m^2)	27.2 ± 3.6	26.9 ± 4.0	0.72
Cardiovascular risk factors			
Hypertension	48 (63.2)	21 (61.8)	0.89
Diabetes mellitus	23 (30.3)	10 (29.4)	0.93
Dyslipidemia	40 (52.6)	12 (35.3)	0.09
Current smoking	37 (48.7)	16 (47.1)	0.82
Family history of coronary artery disease	15 (19.7)	10 (29.4)	0.27
Prior stroke	1 (1.3)	0	0.51
Peripheral artery disease	1 (1.3)	1 (2.9)	0.56
Previous MI	7 (9.2)	3 (8.8)	0.95
Prior PCI	7 (9.2)	3 (8.8)	0.95
Angina before MI	20 (26.3)	7 (20.6)	0.80
Treatment with aspirin prior to MI	12 (15.8)	6 (17.6)	0.85
Renal failure	3 (3.9)	1 (2.9)	0.80
Killip class on admission			
I	69 (90.8)	31 (91.2)	0.96
II	4 (5.3)	2 (5.9)	
III	3 (3.9)	1 (2.9)	
Time delay, (min)			
Onset of chest pain to balloon inflation	197 (145–350)	230 (180–420)	0.25
Loading dose of clopidogrel to blood sampling	70 (48–110)	75 (50–112)	0.41
Lab results on admission			
White blood cells, (× 10^3/µl)	10.7 (9.0–13.7)	11.2 (9.2–13.8)	0.73
Hemoglobin, (g/dl)	14.5 (13.4–15.2)	14.5 (13.1–14.8)	0.45
Hematocrit, (%)	41.8 (39.5–44.0)	42.3 (39.5–44.2)	0.95
Red blood cells, (× 10^6/µl)	4.76 (4.40–5.08)	4.55 (4.20–4.93)	0.15
Platelet count, (× 10^3/µl)	218 (190–271)	230 (184–269)	0.98
Glucose, (mmol/l)	8.4 (7.2–10.3)	7.5 (6.6-9.0)	0.12
Fibrinogen, (g/l)	3.78 (2.99–4.43)	3.40 (2.47–4.23)	0.43
Creatinine, (µmol/l)	94 (78–105)	88 (77–103)	0.55
Total cholesterol, (mmol/l)	5.30 (4.34–6.20)	4.87 (4.22–6.30)	0.96
LDL-cholesterol, (mmol/l)	3.24 (2.48–4.1)	3.09 (2.35–4.10)	0.89
HDL-cholesterol, (mmol/l)	1.10 (0.98–1.32)	1.25 (1.10–1.40)	0.07
Triglycerides, (mmol/l)	1.21 (0.72–1.81)	1.12 (0.70-2.00)	0.95
hs C-reactive protein, (mg/l)	1.96 (1.00-6.23)	2.96 (1.71–28.67)	0.042

Data are expressed as number (percentage), median (interquartile ranges) or mean ± standard deviation

MI myocardial infarction, *PCI* percutaneous coronary intervention, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

Table 2 Antithrombotic pharmacotherapy and invasive procedure

	ICT without polyhedrocytes, $n=76$	ICT with polyhedrocytes, $n=34$	p value
Aspirin, 300 mg p.o.			
Pre-hospital FMC	76 (100)	34 (100)	1.0
Clopidogrel, 600 mg p.o.			0.044
Pre-hospital FMC	48 (63.2)	27 (79.4)	
During PCI	29 (36.8)	6 (20.6)	
Abciximab, i.v.			
During PCI, as a bail-out procedure	21 (27.6)	10 (29.4)	0.85
Unfractionated heparin			
Pre-hospital FMC, 5000 IU i.v.	49 (64.5)	23 (67.6)	0.88
Infarct-related artery			0.37
Left anterior descending	43 (56.6)	16 (47.0)	
Left circumflex	5 (6.6)	4 (11.8)	
Right coronary artery	28 (36.8)	14 (41.2)	
TIMI at baseline			
0/1	69 (90.8)	33 (97.0)	0.46
2	5 (6.6)	1 (3.0)	
3	2 (2.6)	0	
TIMI after aspiration			
0/1	20 (26.3)	3 (8.8)	0.096
2	21 (27.6)	10 (29.4)	
3	35 (46.1)	21 (61.8)	
TIMI after PCI			
0/1	1 (1.3)	1 (3.0)	0.21
2	17 (22.4)	3 (8.8)	
3	58 (76.3)	30 (88.2)	
TMPG after PCI			
0/1	24 (31.6)	6 (17.6)	0.17
2/3	52 (68.4)	28 (82.4)	
Distal embolization during PCI	12 (15.8)	5 (14.7)	0.88
Reference lumen diameter, (mm)	3.40 (2.90–3.40)	2.75 (2.55–3.35)	<0.0001
Reference lumen area, (mm^2)	9.07 (6.60–9.07)	5.94 (5.10–8.29)	<0.0001
Stent size, (mm)	3.5 (3.0–3.5)	3.00 (2.75–3.50)	0.002
Total stent length, (mm)	20 (16–27)	18 (13–23)	0.18
Creatine kinase $_{MAX}$, (IU/l)	2285 (1408–3838)	2656 (1062–5742)	0.67
Isoenzyme MB of creatine kinase, (IU/l)	274 (136–417)	317 (183–480)	0.23
Troponin I_{MAX}, (ng/ml)	46.00 (20.77–105.34)	29.57 (7.39–67.86)	0.14
Left ventricular ejection fraction before discharge, (%)	41 (35–50)	45 (40–50)	0.31

Data are expressed as number (percentage) or median (interquartile range)

FMC first medical contact, *PCI* percutaneous coronary intervention, *TIMI* Thrombolysis in myocardial infarction, *TMPG* TIMI myocardial perfusion grade, *CK-MB$_{MAX}$* isoenzyme MB of creatine kinase

Polyhedrocyte content was moderately correlated with erythrocyte content ($r=0.32$, $p=0.001$) and inversely correlated with platelet content ($r=-0.28$, $p=0.006$). There was no correlation between fibrin and polyhedrocyte content in ICT. Similar findings were observed in the polyhedrocyte-rich thrombi, in which erythrocyte content ($p<0.05$) was larger, platelet content ($p<0.001$) lower and

fibrin content ($p=0.79$) similar as compared with patients without polyhedrocytes in ICT.

Patient characteristics and polyhedrocytes in ICT

Demographic variables and cardiovascular risk factors were not associated with polyhedrocyte presence and content in ICT (Table 1, Supplementary Table 1).

A pre-hospital bolus of UFH also did not influence polyhedrocyte content (Table 2). However, in 75 (68.2%) patients who had been pretreated with loading dose of clopidogrel before hospital admission, polyhedrocytes were found more frequently (36% vs. 17.1%, $p=0.044$). Also median content of polyhedrocytes in those patients was higher as compared with individuals not pretreated with clopidogrel (16.1 ± 13.4 vs. 5.2 ± 6.2%, $p=0.022$). Moreover, ICT rich in polyhedrocytes were found only in patients pre-loaded with clopidogrel (14.7% vs. 0%, $p=0.017$). Patients pretreated with clopidogrel had higher content of fibrin in ICT (58 [41–75] vs. 52 [31–61] %, $p<0.05$).

There was no association between the presence or content of polyhedrocytes in ICT and baseline laboratory investigations including red blood cell count, platelet count, and fibrinogen (Supplementary Table 1). In patients who formed polyhedrocytes, CRP was higher as compared with the remainder (3.4 [1.7–35.2] vs. 2.0 [1.0–6.2] ng/ml, respectively, $p=0.042$). CRP was correlated with polyhedrocytes content in ICT ($r=0.29$, $p=0.01$). Fibrinogen but not CRP was positively correlated with fibrin content ($r=0.30$, $p<0.01$) and inversely associated with erythrocyte content ($r=-0.33$, $p=0.004$) in ICT.

Fibrin clot properties were measured in a subgroup of 71 patients. There was no correlation between Ks or t50% and polyhedrocytes content in ICT (Supplementary Fig. 1). Moreover, there were no differences regarding Ks ($p=0.38$) and t50% ($p=0.97$) between patients with polyhedrocyte-rich thrombi and those with lower polyhedrocytes content in ICT.

The longer time of ischemia the higher number of FoV with polyhedrocytes. The time from onset of chest pain to balloon inflation was positively correlated with the polyhedrocyte content in the ICT ($r=0.26$, $p=0.007$) (Fig. 2a). Polyhedrocytes were present in ICT of 9 (20.9%) patients with time of ischemia of ≤ 3 h and in 25 (37.3%) patients with ischemic time of >3 h ($p=0.09$). Time of ischemia was also longer in patients with ICT rich in polyhedrocytes as compared with those with <50% of FoV covered by polyhedrocytes (360 [200–480] vs. 208 [148–350] min, respectively, $p=0.023$). Of 11, 10 patients with polyhedrocyte-rich ICT had time of ischemia of >3 h. Longer time of ischemia was also associated with higher content of fibrin ($r=0.27$, $p=0.005$) and a non-significant trend to lower content of erythrocytes ($r=-0.19$, $p=0.063$) in ICT.

Infarct-related artery characteristics and polyhedrocytes in ICT

Both median values of mRLD and minRLD were inversely correlated with polyhedrocyte content in the ICT ($r=-0.44$ and $r=-0.53$, respectively, $p<0.0001$ for both) (Fig. 2b). A median value of minRLD was lower by 20% ($p<0.0001$) in patients with polyhedrocytes present in ICT as compared with the remainder (2.73 [2.40–3.23] vs. 3.40 [2.90–3.40] mm, respectively) (Fig. 2c). MinRLD was significantly lower in patients with polyhedrocyte-rich ICT as compared with subjects with <50% FoV covered by polyhedrocytes (2.50 [2.20–2.70] vs. 3.30 [2.90–3.40] mm, respectively, $p<0.0001$) (Fig. 2d).

The smaller IRA reference lumen area, the higher polyhedrocytes content ($r=-0.43$, $p<0.0001$) (Fig. 2b). The median RLA was lower in patients who formed polyhedrocytes in ICT by 31% ($p<0.0001$) as compared with the remaining subjects (6.26 [5.03–8.52] vs. 9.05 [6.58–9.05] mm, respectively) (Fig. 2c). The RLA was also lower in polyhedrocyte-rich ICT as compared with patients with lower number of FoV with polyhedrocytes [5.08 (4.61–5.94) vs. 8.76 (6.58–9.05) mm, respectively, $p<0.0001$] (Fig. 2d). Similar relationships were found for stent diameter (Fig. 2b–d).

The content of fibrin, erythrocytes and platelets in ICT was not associated with mRLD, minRLD, RLA, stent diameter and its length (all $p>0.25$ for each relationship).

Clinical outcomes and myocardial reperfusion

Within 30-day follow-up, two patients died, three had recurrent MI and 17 (15.5%) required hospitalization due to symptoms of heart failure. In five patients who died or had recurrent MI within 30-day follow-up, a median polyhedrocyte content was similar as compared with patients without these endpoints ($p=0.73$).

Patients with and without polyhedrocytes in ICT did not differ significantly in terms of final epicardial TIMI-3 flow (88.2 vs. 76.3%, $p=0.10$). However, the higher number of patients with polyhedrocyte-rich ICT the better TIMI myocardial perfusion grade after the whole procedure ($p=0.022$, Fig. 3a). There was a tendency in favor of patients with higher polyhedrocyte content in ICT concerning better restored epicardial blood flow immediately after ICT aspiration ($p=0.06$, Fig. 3b) and myocardial perfusion after the whole PCI ($p=0.07$, Fig. 3c). In patients with time of ischemia of more than 3 h ($n=67$), polyhedrocytes presence in ICT was associated with more frequent TIMI-2/3 flow after thrombus aspiration (96% vs. 67%, $p=0.02$) and TIMI-2/3 myocardial perfusion grade after PCI (92% vs. 57%, $p=0.044$) as compared with patients without

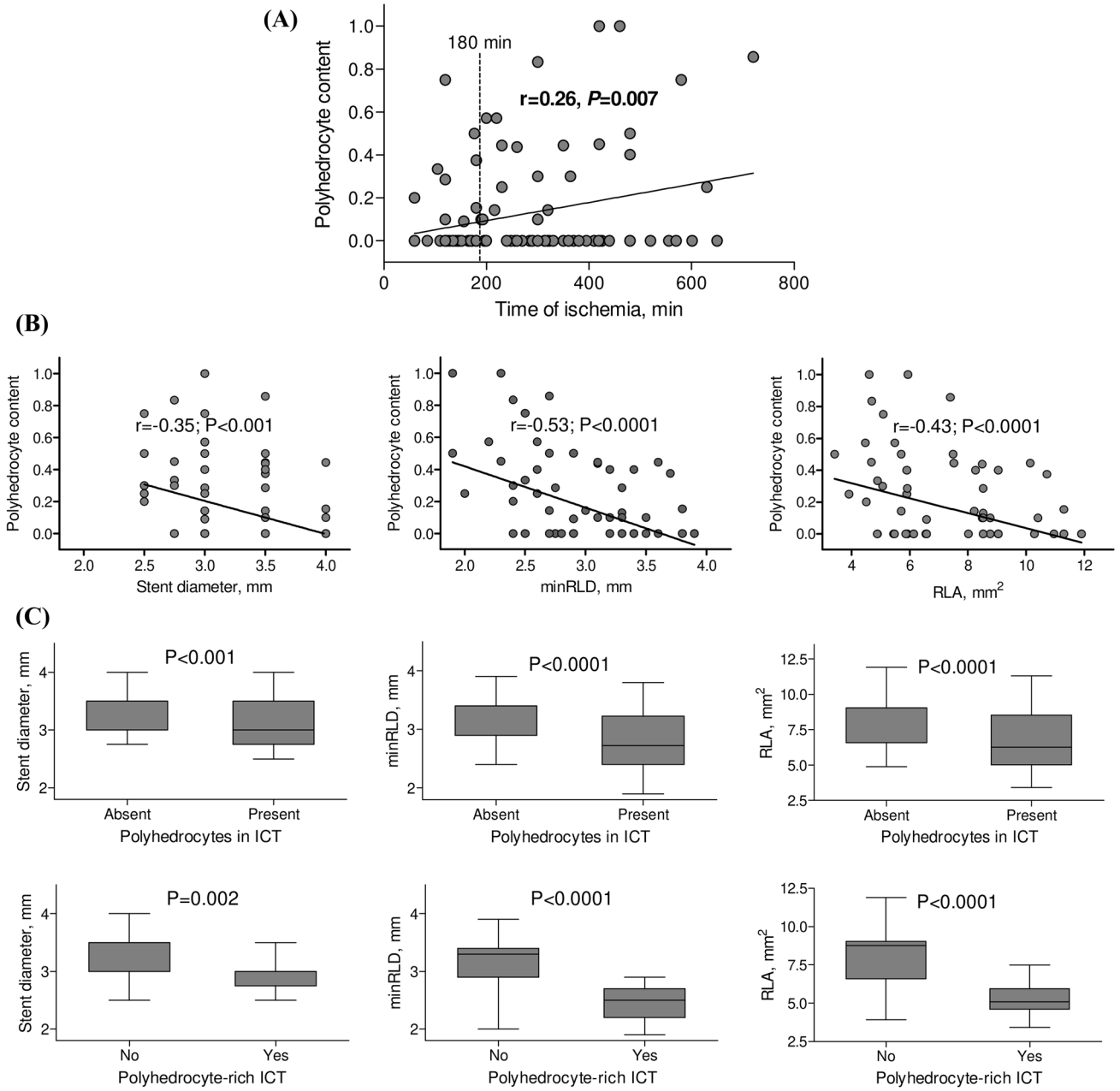

Fig. 2 Polyhedrocyte presence or content in intracoronary thrombus (ICT) versus time of ischemia, infarct-related artery lumen diameter, area and stent diameter. Prolonged time of ischemia is associated with higher content of polyhedrocytes (**a**). The lower IRA lumen area or diameter, the higher polyhedrocyte content in the ICT (**b**–**d**). The polyhedrocyte content in the ICT is expressed as a ratio. *IRA* infarct-related artery, *minRLD* minimal reference lumen diameter of IRA, *RLA* reference lumen area of IRA

polyhedrocytes in ICT (Fig. 4). Similar findings were not observed in patients with time of ischemia of ≤ 3 h.

Predictors of polyhedrocyte content in ICT

The multivariable model for polyhedrocyte content in ICT is shown in Table 3. The independent variables identified as associated ($p < 0.2$) with polyhedrocyte content in ICT in univariate model (Tables 1, 2; Fig. 2, Supplementary Table 1) were included to the multivariate regression model. Moreover, significant correlations between independent variables including mRLD, minRLD, RLA and stent diameter (for all $r > 0.95$, $p < 0.0001$), dyslipidemia and red blood cells ($r = 0.31$, $p = 0.001$), CRP and time of ischemia ($r = 0.28$, $p = 0.005$), HDL-cholesterol and time of ischemia ($r = 0.24$, $p = 0.017$) were found. In a final multivariable

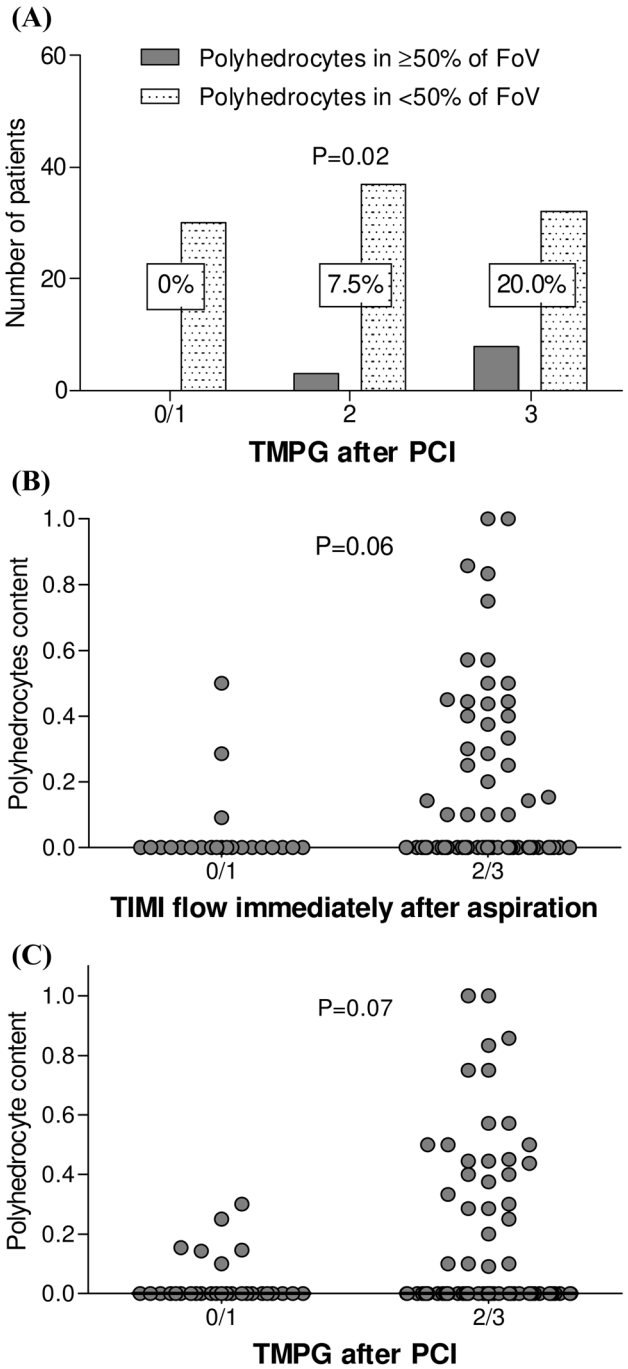

Fig. 3 Polyhedrocyte content in the intracoronary thrombus (ICT) and reperfusion in angiography. Higher polyhedrocyte content in the ICT was in favor of better epicardial blood flow immediately after aspiration thrombectomy (**b**) and better myocardial perfusion after PCI (**a, c**). The polyhedrocyte content in the ICT is expressed as a ratio. In **a**, percentages were calculated as the number of patients with polyhedrocyte-rich ICT divided by the number of all patients with the same final perfusion and multiplied by 100% (for TMPG-0/1 − 0/30, for TMPG-2–3/40 and for TMPG-3–8/40 respectively). *FoV* field of view, *TIMI* thrombolysis in myocardial infarction, *TMPG* TIMI myocardial perfusion grades, *PCI* percutaneous coronary intervention

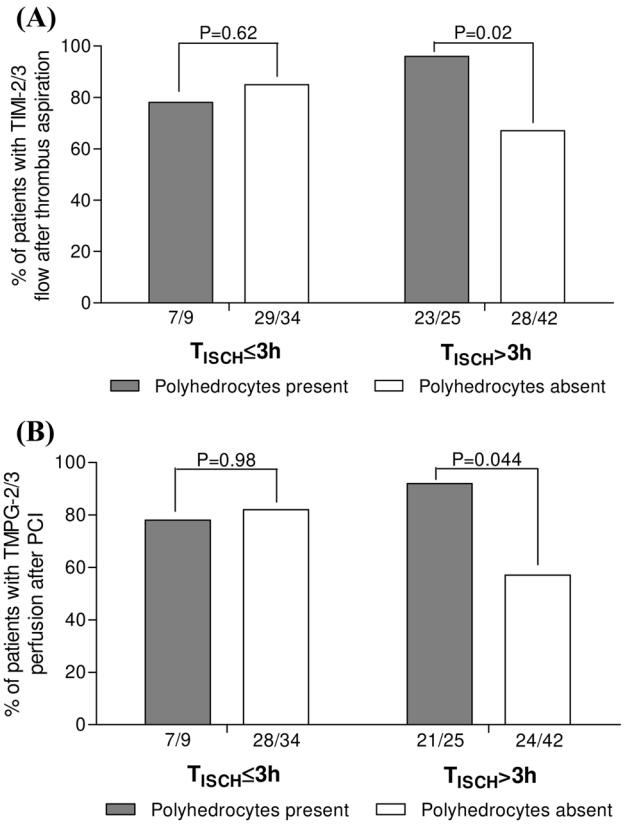

Fig. 4 The epicardial and myocardial reperfusion in relation to polyhedrocytes presence in the intracoronary thrombus and time of ischemia. In patients with time of ischemia of more than 3 h, TIMI-2/3 flow immediately after thrombus aspiration and final TIMI-2/3 myocardial perfusion grades after PCI were more frequent in subjects with polyhedrocytes present in ICT. *TIMI* Thrombolysis in myocardial infarction, *TMPG* TIMI myocardial perfusion grades, *PCI* percutaneous coronary intervention, T_{ISCH} time of ischemia

model, minimal IRA reference lumen diameter and time of ischemia independently affected polyhedrocyte content in the ICT with variance of $R^2 = 0.45$ ($p < 0.0001$) (Table 3).

Discussion

The current study is the first to demonstrate that both local vascular factors and clinical conditions affect the formation of polyhedrocytes in the intracoronary thrombus aspirated from the IRA in STEMI patients within 12 h since chest pain onset. To our knowledge, this is the largest study in which the polyhedrocyte content in ICT was analyzed in detail. We identified factors that affect polyhedrocyte formation in vivo in epicardial arteries in acute MI, namely narrow infarct-related artery and late presentation of MI. Importantly, in patients with time of ischemia of more than 3 h, polyhedrocytes presence in ICT was associated with significantly better epicardial blood flow following thrombus aspiration

Table 3 Multivariable analysis with polyhedrocytes in intracoronary thrombus as a dependent variable

Polyhedrocyte content in ICT	Independent variables	β	95% CI for β		p value
$R^2 = 0.45$ $F(4106) = 21.80$ $p < 0.0001$	Minimal IRA reference lumen diameter (per 1 mm)	−0.50	−0.55	−0.44	< 0.0001
	Time of ischemia (per 1 min)	0.20	0.16	0.24	0.035
	Clopidogrel loading (no/yes)	0.13	0.08	0.18	0.12
	Stent length (per 1 mm)	−0.14	−0.20	−0.07	0.45

ICT intracoronary thrombus, *CI* confidence interval, R^2 variance, *r* regression coefficients

and better myocardial perfusion after the whole procedure of primary PCI.

Our findings suggest that polyhedrocyte formation and clot contraction are modulated by modifiable and unmodifiable factors, which expands the current knowledge on clot compression in arteries based largely on ex vivo studies. In a model of in vitro formed and then contracted whole blood clots obtained from the healthy volunteers without antiplatelet therapy [15], it has been found that the inner part of the clot is composed of closely packed polyhedral erythrocytes with little fibrin and few platelets. In turn, the outer surface of the contracted clots was composed primarily of a dense meshwork of fibrin together with platelet aggregates. Similar findings have been found for STEMI patients [18]. In our study, we have shown that after aspirin administration and on clopidogrel in two-third of cases at the time of ICT aspiration from infarct-related artery, almost one-third of thrombi contained polyhedrocytes, including one-tenth of ICT, in which polyhedral erythrocytes covered of more than 50% of fields of view. Our findings suggest that in ICT in which its size is limited by the diameter of IRA lumen, polyhedrocyte formation is the most pronounced in smaller epicardial arteries as compared with those of larger diameter. Moreover, it is possible that polyhedrocyte formation might be enhanced in MI by arterial wall contraction with the pressure inversely proportional to the artery diameter according to the Laplace's law. This suggests the important role of vascular factors in thrombus compression in vivo.

As expected, we confirmed that the time of ischemia is important predictor of intracoronary thrombus composition and longer ischemia is associated with lower content of platelets and higher content of fibrin [18] and also polyhedrocytes. In vitro experiments indicate that shortly after clotting erythrocytes were biconcave, but as contraction occurred, biconcave erythrocytes became polyhedral [16]. We found that prolonged time of ischemia was also accompanied by higher plasma CRP. In turn, it was shown that CRP binds to fibrinogen and thus may modify fibrin formation [23, 24].

Clot contraction is dynamic and multifactorial process which is enhanced by thrombin, high platelet counts, platelet–fibrin interactions, factor XIIIa cross-linking and activation of platelet myosin enhance clot contraction, whereas high fibrinogen, high hematocrit and increased red blood cell rigidity have the opposite effect [16]. Contrary to in vitro observations [15, 16], we did not find any relationships between polyhedrocyte presence or content in ICT and any of the three variables, i.e., hematocrit, red blood cell count and plasma fibrinogen concentration in vivo (all concentrations above 2.5 g/l). This indicates that intravascular thrombus composition in STEMI does not simply reflect erythrocyte count and fibrinogen levels at the time of acute ischemia. Despite our previous findings showing that ex vivo measured fibrin clot properties are independently associated with ICT structure [18], we did not find any relationships between polyhedrocytes content in ICT and fibrin clot permeability or clot lysis time. Regarding platelet count, it is known that if platelet count is < 75 or $125–150 \times 10^3/\mu l$, clots made ex vivo did not contract or poorly contracted, respectively [16]. Our SEM images showed that in vivo platelets (despite the use of antiplatelet agents) still support fibrin formation in ICT and are involved in contraction.

Contraction of the blood clot formed in vitro from stroke patients was significantly reduced as compared with healthy subjects [20]. It has been postulated that clinical consequences of clot contraction are beneficial by increasing blood flow in the obstructed vessel. In our patients with higher content of polyhedrocytes in the ICT, it was easier to restore complete epicardial blood flow immediately following aspiration thrombectomy and as a result also myocardial perfusion after the whole procedure. Different demographic [25, 26], laboratory [27–31], ECG [32, 33], associated with cardiac function [34–37] and treatment [38–42] factors influence the immediate and long-term outcomes in myocardial infarction. We found that, in patients with time of ischemia of more than 3 h, polyhedrocytes presence in ICT was associated with significantly better angiographic outcome. It is likely that well contracted thrombi are more effectively aspirated from IRA during primary angioplasty and therefore novel therapies altering cot compression might be useful in acute phase of MI. Previous findings showed that erythrocyte-rich thrombi were linked with impaired myocardial reperfusion in STEMI patients [43] and histo-pathologically classified as old intracoronary thrombi predicted 1-year mortality after STEMI [44]. Long-term follow-up is needed to assess clinical endpoints in relation to thrombus

compression in STEMI patients treated as recommended in the present guidelines.

In the current study, scanning electron microscopy as a reference method for the demonstration of morphological features of polyhedrocytes was used. In turn, confocal light microscopy with an optical clearing method termed cCLOT enables intravital imaging of structures deep within clots, however, with lower resolution. Recently, also T2-weighted technique of magnetic resonance enabled to follow the dynamic process of clot formation and effects of forces generated during contraction of erythrocytes [15].

Our study has several limitations. First, the sample size and the number of clinical adverse events are not enough to draw clinical conclusions, but the study was adequately powered for surrogate endpoints. We are aware of the fact that associations do not necessarily mean the cause–effect relationship. Second, during primary PCI, thrombus aspirations often are not complete and not all of the thrombi components are available for the analysis. Therefore, the removed material may not be representative for the whole intracoronary thrombus. Third, platelet reactivity was not measured and the method for the detection of polyhedral erythrocytes was only semiquantitative, however, inter-observer and intra-observer agreement was high. Finally, we cannot exclude that patients with no or lower polyhedrocytes content in ICT might have developed a higher number of polyhedral erythrocytes if the intervention would have been performed later.

In conclusion, our findings demonstrate that during acute phase of STEMI, polyhedrocytes in the intracoronary thrombi are formed in a large amount in the narrow infarct-related artery, in subjects with prolonged time of ischemia, and their presence in patients with late MI presentation is associated with more effective thrombus aspiration and better myocardial reperfusion. Our study shows that in contrast to ex vivo findings, intravascular thrombus composition, including polyhedrocytes, is subjected to a potent impact of local and disease-specific factors. Further in vivo studies on a role of polyhedrocytes in thromboembolic disorders are needed to elucidate their clinical relevance.

Acknowledgements This work was supported by grants of the Jagiellonian University Medical College (K/ZDS/006441) and by National Science Centre Poland (2016/21/B/NZ5/01378).

Compliance with ethical standards

Conflict of interest The authors have nothing to disclose in relation to this study.

References

1. Dégano IR, Salomaa V, Veronesi G, Ferriéres J, Kirchberger I, Laks T, Havulinna AS, Ruidavets JB, Ferrario MM, Meisinger C, Elosua R, Marrugat J (2015) Twenty-five-year trends in myocardial infarction attack and mortality rates, and case-fatality, in six European populations. Heart 101:1413–1421. https://doi.org/10.1136/heartjnl-2014-307310
2. Fox KA, Dabbous OH, Goldberg RJ, Pieper KS, Eagle KA, Van de Werf F, Avezum A, Goodman SG, Flather MD, Anderson FA Jr, Granger CB (2006) Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). BMJ 333:1091. https://doi.org/10.1136/bmj.38985.646481.55
3. Roffi M, Patrono C, Collet JP et al (2016) 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 37:267–315. https://doi.org/10.1093/eurheartj/ehv320
4. Nührenberg TG, Hochholzer W, Mashayekhi K, Ferenc M, Neumann FJ (2018) Efficacy and safety of bivalirudin for percutaneous coronary intervention in acute coronary syndromes: a meta-analysis of randomized-controlled trials. Clin Res Cardiol 107:807–815. https://doi.org/10.1007/s00392-018-1251-1
5. Fournier S, Muller O, Benedetto U, Roffi M, Pilgrim T, Eberli FR, Rickli H, Radovanovic D, Erne P, Cook S, Noble S, Fesselet R, Zuffi A, Degrauwe S, Masci P, Windecker S, Eeckhout E, Iglesias JF, on behalf on the AMIS Plus Investigators (2018) Circadian dependence of manual thrombus aspiration benefit in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Clin Res Cardiol 107:338–346. https://doi.org/10.1007/s00392-017-1189-8
6. Khoury S, Carmon S, Margolis G, Keren G, Shacham Y (2017) Incidence and outcomes of early left ventricular thrombus following ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. Clin Res Cardiol 106:695–701. https://doi.org/10.1007/s00392-017-1111-4
7. Undas A, Zalewski J, Krochin M, Siudak Z, Sadowski M, Pregowski J, Dudek D, Janion M, Witkowski A, Zmudka K (2010) Altered plasma fibrin clot properties are associated with in-stent thrombosis. Arterioscler Thromb Vasc Biol 30:276–282. https://doi.org/10.1161/ATVBAHA.109.194936
8. Henriques JP, Zijlstra F, Ottervanger JP, de Boer MJ, van 't Hof AW, Hoorntje JC, Suryapranata H (2002) Incidence and clinical significance of distal embolization during primary angioplasty for acute myocardial infarction. Eur Heart J 23:1112–1117. https://doi.org/10.1053/euhj.2001.3035
9. Zalewski J, Zmudka K, Musialek P, Zajdel W, Pieniazek P, Kadzielski A, Przewlocki T (2004) Detection of microvascular injury by evaluating epicardial blood flow in early reperfusion following primary angioplasty. Int J Cardiol 96:389–396. https://doi.org/10.1016/j.ijcard.2003.08.009
10. Zalewski J, Undas A, Godlewski J, Stepien E, Zmudka K (2007) No-reflow phenomenon after acute myocardial infarction is associated with reduced clot permeability and susceptibility to lysis. Arterioscler Thromb Vasc Biol 27:2258–2265. https://doi.org/10.1161/ATVBAHA.107.149633
11. Stone GW, Peterson MA, Lansky AJ, Dangas G, Mehran R, Leon MB (2002) Impact of normalized myocardial perfusion after successful angioplasty in acute myocardial infarction. J Am Coll Cardiol 39:591–597. https://doi.org/10.1016/S0735-1097(01)01779-X
12. Zalewski J, Bogaerts K, Desmet W, Sinnaeve P, Berger P, Grines C, Danays T, Armstrong P, Van de Werf F (2011) Intraluminal thrombus in facilitated versus primary percutaneous coronary intervention; an angiographic substudy of the ASSENT-4 PCI (assessment of the safety and efficacy of a new treatment strategy

with percutaneous coronary intervention) trial. J Am Coll Cardiol 57:1867–1873. https://doi.org/10.1016/j.jacc.2010.10.061
13. Badimon L, Chesebro JH, Badimon JJ (1992) Thrombus formation on ruptured atherosclerotic plaques and rethrombosis on evolving thrombi. Circulation 86(6 Suppl):III74–I85
14. Furie B, Furie BC (2008) Mechanisms of thrombus formation. N Engl J Med 359:938–949. https://doi.org/10.1056/NEJMra0801082
15. Cines DB, Lebedeva T, Nagaswami C, Hayes V, Massefski W, Litvinov RI, Rauova L, Lowery TJ, Weisel JW (2014) Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. Blood 123:1596–1603. https://doi.org/10.1182/blood-2013-08-523860
16. Tutwiler V, Litvinov RI, Lozhkin AP, Peshkova AD, Lebedeva T, Ataullakhanov FI, Spiller KL, Cines DB, Weisel JW (2016) Kinetics and mechanics of clot contraction are governed by the molecular and cellular composition of the blood. Blood 127:149–159. https://doi.org/10.1182/blood-2015-05-647560
17. Zabczyk M, Sadowski M, Zalewski J, Undas A (2015) Polyhedrocytes in intracoronary thrombi from patients with ST-elevation myocardial infarction. Int J Cardiol 179:186–187. https://doi.org/10.1016/j.ijcard.2014.10.004
18. Zalewski J, Bogaert J, Sadowski M, Woznicka O, Doulaptsis K, Ntoumpanaki M, Ząbczyk M, Nessler J, Undas A (2015) Plasma fibrin clot phenotype independently affects intracoronary thrombus ultrastructure in patients with acute myocardial infarction. Thromb Haemost 113:1258–1269. https://doi.org/10.1160/TH14-09-0801
19. Litvinov RI, Weisel JW (2017) Role of red blood cells in haemostasis and thrombosis. ISBT Sci Ser 12:176–183. https://doi.org/10.1111/voxs.12331
20. Tutwiler V, Peshkova AD, Adrianova IA, Khasanova DR, Weisel JW, Litvinov RI (2017) Contraction of blood cells is impaired in acute ischemic stroke. Arterioscler Thromb Vasc Biol 37:271–279. https://doi.org/10.1161/ATVBAHA.116.308622
21. Silvain J, Collet JP, Nagaswami C, Beygui F, Edmondson KE, Bellemain-Appaix A, Cayla G, Pena A, Brugier D, Barthelemy O, Montalescot G, Weisel JW (2011) Composition of coronary thrombus in acute myocardial infarction. J Am Coll Cardiol 57:1359–1367. https://doi.org/10.1016/j.jacc.2010.09.077
22. Collins TJ (2007) ImageJ for microscopy. Biotechniques 43:25–30. https://doi.org/10.2144/000112517
23. Undas A, Szułdrzynski K, Stepien E, Zalewski J, Godlewski J, Tracz W, Pasowicz M, Zmudka K (2008) Reduced clot permeability and susceptibility to lysis in patients with acute coronary syndrome: effects of inflammation and oxidative stress. Atherosclerosis 196:551–557. https://doi.org/10.1016/j.atherosclerosis.2007.05.028
24. Salonen EM, Vartio T, Hedman K (1984) Binding of fibronectin by the acute phase C-reactive protein. J Biol Chem 259:1496–1501
25. Ek A, Ekblom Ö, Hambraeus K, Cider Å, Kallings LV, Börjesson M (2018) Physical inactivity and smoking after myocardial infarction as predictors for readmission and survival: results from the SWEDEHEART-registry. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1360-x. **[Epub ahead of print]**
26. Fang XY, Spieler D, Albarqouni L, Ronel J, Ladwig KH (2018) Impact of generalized anxiety disorder (GAD) on prehospital delay of acute myocardial infarction patients. Findings from the multicenter MEDEA study. Clin Res Cardiol 107:471–478. https://doi.org/10.1007/s00392-018-1208-4
27. Groot HE, Al Ali L, van der Horst ICC, Schurer RAJ, van der Werf HW, Lipsic E, van Veldhuisen DJ, Karper JC, van der Harst P (2018) Plasma interleukin 6 levels are associated with cardiac function after ST-elevation myocardial infarction. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1387-z. **[Epub ahead of print]**
28. Bodde MC, Hermans MC, Jukema MPJ, Schalij JW, Lijfering MJ, Rosendaal WM, Romijn FR, Ruhaak FPHTM, van der Laarse LR, Cobbaert A (2018) Apolipoproteins A1, B, and apoB/apoA1 ratio are associated with first ST-segment elevation myocardial infarction but not with recurrent events during long-term follow-up. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1381-5. **[Epub ahead of print]**
29. Ferreira JP, Barros A, Pitt B, Montalescot G, de Sa EL, Hamm CW, Flather M, Verheugt F, Shi H, Leite-Moreira A, Vincent J, Rossignol P, Zannad F (2018) Collagen biomarker bioprofiles predicting the antifibrotic response to eplerenone in myocardial infarction: findings from the REMINDER trial. Clin Res Cardiol 107:1192–1195. https://doi.org/10.1007/s00392-018-1373-5
30. Plakht Y, Gilutz H, Shiyovich A (2018) Sodium levels during hospitalization with acute myocardial infarction are markers of in-hospital mortality: Soroka acute myocardial infarction II (SAMI-II) project. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1268-5. **[Epub ahead of print]**
31. Cecchi E, Parodi G, Fatucchi S, Angelotti P, Giglioli C, Gori AM, Bandinelli B, Bellandi B, Sticchi E, Romagnuolo I, Mannini L, Antoniucci D, Abbate (2016) Prevalence of thrombophilic disorders in takotsubo patients: the (ThROmbophylia in TAkotsubo cardiomyopathy) TROTA study. Clin Res Cardiol 105:717–726. https://doi.org/10.1007/s00392-016-0977-x. **[Epub ahead of print]**
32. Reithmann C, Fiek M, Aynur Z, Ulbrich M (2018) Electrocardiographic criteria of epicardial ventricular tachycardia with anterior origin. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1349-5. **[Epub ahead of print]**
33. Gassanov N, Le MT, Caglayan E, Hellmich M, Erdmann E, Er F (2018) Novel ECG-based scoring tool for prediction of takotsubo syndrome. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1314-3. **[Epub ahead of print]**
34. Stiermaier T, Pöss J, Eitel C, de Waha S, Fuernau G, Desch S, Thiele H, Eitel I (2018) Impact of left ventricular hypertrophy on myocardial injury in patients with ST-segment elevation myocardial infarction. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1273-8. **[Epub ahead of print]**
35. Khoury S, Steinvil A, Gal-Oz A, Margolis G, Hochstatd A, Topilsky Y, Keren G, Shacham Y (2018) Association between central venous pressure as assessed by echocardiography, left ventricular function and acute cardio-renal syndrome in patients with ST segment elevation myocardial infarction. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1266-7. **[Epub ahead of print]**
36. Abdin A, Pöss J, Fuernau G, Ouarrak T, Desch S, Eitel I, de Waha S, Zeymer U, Böhm M, Thiele H (2018) Correction to: prognostic impact of baseline glucose levels in acute myocardial infarction complicated by cardiogenic shock-a substudy of the IABP-SHOCK II-trial. Clin Res Cardiol 107:531. https://doi.org/10.1007/s00392-018-1225-3
37. Abdin A, Pöss J, Fuernau G, Ouarrak T, Desch S, Eitel I, de Waha S, Zeymer U, Böhm M, Thiele H (2018) Revision: prognostic impact of baseline glucose levels in acute myocardial infarction complicated by cardiogenic shock-a substudy of the IABP-SHOCK II-trial. Clin Res Cardiol 107:517–523. https://doi.org/10.1007/s00392-018-1213-7. **(PMID: 29423774)**
38. Backhaus T, Fach A, Schmucker J, Fiehn E, Garstka D, Stehmeier J, Hambrecht R, Wienbergen H (2018) Management and predictors of outcome in unselected patients with cardiogenic shock complicating acute ST-segment elevation myocardial infarction: results from the Bremen STEMI Registry. Clin Res Cardiol 107:371–379. https://doi.org/10.1007/s00392-017-1192-0
39. Ferenc M, Banholzer N, Hochholzer W, Mashayekhi K, Comberg T, Rothe J, Valina CM, Toma A, Löffelhardt N, Gick M, Neumann FJ, Nührenberg TG (2018) Long-term results after PCI of unprotected distal left main coronary artery stenosis: the Bifurcations

Bad Krozingen (BBK)-Left Main Registry. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1337-9. **[Epub ahead of print]**

40. Richardt G, Maillard L, Nazzaro MS, Abdel-Wahab M, Carrié D, Iñiguez A, Garot P, Abdellaoui M, Morice MC, Foley D, Copt S, Stoll HP, Urban P (2018) Polymer-free drug-coated coronary stents in diabetic patients at high bleeding risk: a pre-specified sub-study of the LEADERS FREE trial. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1308-1. **[Epub ahead of print]**
41. Feistritzer HJ, Desch S, de Waha S, Jobs A, Zeymer U, Thiele H (2018) German contribution to development and innovations in the management of acute myocardial infarction and cardiogenic shock. Clin Res Cardiol. https://doi.org/10.1007/s00392-018-1276-5. **[Epub ahead of print]**
42. Tripodi A, Braham S, Scimeca B, Moia M, Peyvandi F (2018) How and when to measure anticoagulant effects of direct oral anticoagulants? Practical issues. Pol Arch Intern Med 128:379–385. https://doi.org/10.20452/pamw.4287
43. Yunoki K, Naruko T, Sugioka K, Inaba M, Iwasa Y, Komatsu R, Itoh A, Haze K, Inoue T, Yoshiyama M, Becker AE, Ueda M (2012) Erythrocyte-rich thrombus aspirated from patients with ST-elevation myocardial infarction: association with oxidative stress and its impact on myocardial reperfusion. Eur Heart J 33:1480–1490. https://doi.org/10.1093/eurheartj/ehr486
44. Li X, Kramer MC, Damman P, van der Wal AC, Grundeken MJ, van Straalen JP, Koch KT, Henriques JP, Baan J Jr, Vis MM, Piek JJ, Fischer JC, Tijssen JG, de Winter RJ (2016) Older coronary thrombus is an independent predictor of 1-year mortality in acute myocardial infarction. Eur J Clin Invest 46:501–510. https://doi.org/10.1111/eci.12619