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Monocyte-platelet aggregates affect local inflammation in patients with acute myocardial infarction☆

Hans Kossmann [a,1], Christoph Rischpler [b,2], Franziska Hanus [a], Stephan G. Nekolla [b,3], Karl P. Kunze [b], Katharina Götze [c], Alexander Goedel [a], Hendrik Sager [d], Adnan Kastrati [d,3], Daniel Sinnecker [a,3], Christian Kupatt [a,3], Tareq Ibrahim [a], Markus Schwaiger [b,3], Karl-Ludwig Laugwitz [a,3], Ralf J. Dirschinger [a,*,1]

[a] Klinik und Poliklinik für Innere Medizin I, Klinikum rechts der Isar, Technische Universität München, Munich, Germany
[b] Nuklearmedizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany
[c] Klinik und Poliklinik für Innere Medizin III, Klinikum rechts der Isar, Technische Universität München, Munich, Germany
[d] Deutsches Herzzentrum, Technische Universität München, Munich, Germany

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ABSTRACT

The local inflammatory response following acute myocardial infarction (AMI) is increasingly being recognized as a central factor determining infarct healing. Myocardial inflammation can be visualized in patients using fasting ^{18}F-FDG PET/MRI. Although this novel biosignal correlates with long-term functional outcome, the corresponding cellular substrate is not well understood. Here we present a retrospective analysis of 29 patients with AMI who underwent revascularization, suggesting a connection between post infarction myocardial fasting ^{18}F-FDG uptake, monocyte platelet aggregates (MPA), and $P2Y_{12}$ inhibition. In detail, patients with high MPA percentages of $CD14^{high}CD16^{+}$ and $CD14^{low}CD16^{+}$ monocytes had significantly higher local ^{18}F-FDG uptake (SUV_{mean}) in the infarcted myocardium than patients with low MPA ($p < 0.05$). Furthermore, there was an association of high MPA percentage in all monocyte subpopulations with deteriorating ΔLV-EF after 6 months ($p < 0.01$), which was confirmed in an extended analysis with additional 29 patients without PET/MRI data available. In this analysis, administration of Ticagrelor was associated with lower MPA percentage of $CD14^{high}$ monocyte subpopulations than Clopidogrel ($p < 0.01$) or Prasugrel ($p < 0.05$).
Taken together, the findings from this analysis suggest that platelet aggregability may affect monocyte extravasation into the infarcted myocardium and influence long-term functional outcome. $P2Y_{12}$ inhibition may intervene in this pathophysiologic process. Prospective studies are needed to further examine this important relationship.

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1. Introduction

In the past years, the role of the immune system in cardiac remodeling after acute myocardial infarction (AMI) has gained increasing interest as potential therapeutic target. AMI triggers the release and local extravasation of leukocytes, including monocyte subsets, which play a central role in the initiation, expansion, and resolution of myocardial inflammation [1–4]. In humans, the initial extravasation of neutrophils is followed by $CD14^{high}$ $CCR2^{+}$ inflammatory monocytes (Ly-$6C^{high}$ in mice). After 72 h, the injured heart switches to a CX3CL1-mediated recruitment of *anti-inflammatory* $CD14^{low}$ $CD16^{+}$ monocytes (Ly-$6C^{low}$ in mice) [5,6]. Adequate infarct healing requires a coordinated cell recruitment with balanced pro- and anti-inflammatory factors [7,8]. In humans, circulating $CD14^{+}$ monocyte counts – particularly $CD14^{high}$ $CD16^{+}$ monocytes (termed *intermediate monocytes* by some authors) correlate with increased recurrent cardiovascular events [9] and adverse remodeling [2] after AMI.

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* Corresponding author at: Klinik und Poliklinik für Innere Medizin I, Technische Universität München, Klinikum rechts der Isar, Ismaninger Str. 22, 81675 Munich, Germany.
E-mail address: r.dirschinger@tum.de (R.J. Dirschinger).
[1] These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.
[2] Current affiliation: Klinikum für Nuklearmedizin, Universitätsklinikum Essen, Germany.
[3] DZHK (Deutsches Zentrum für Herz-Kreislauf-Forschung e.V.), partner site Munich Heart Alliance, Munich, Germany.

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A preclinical rodent PET study presented evidence that post-AMI ^{18}F-FDG uptake reflects the monocytic inflammatory response in the myocardium [10]. We have previously shown that this strategy can be clinically translated, enabling visualization of the myocardial inflammation in patients with AMI using ^{18}F-FDG PET/MRI [10,11]. Transient suppression of glucose uptake by cardiomyocytes is required to detect leukocytes in the heart, which can be accomplished by low-carb diet, fasting, and administration of heparin before imaging [12,13]. In our previous study, the intensity of ^{18}F-FDG uptake 5 days after AMI inversely correlated with the change of left ventricular function after 6 months, underlining the importance of these findings. Interestingly, the local intensity of ^{18}F-FDG uptake in the myocardium did not reflect monocyte or other leucocyte counts [11], suggesting that local transmigration of monocytes into the infarct area is mainly regulated by other factors, such as crosstalk with other leukocyte subsets, endothelial cells, local cytokine gradients, and platelet-leukocyte interaction.

Among leukocytes, monocytes show the highest aggregation with platelets due to their high affinity for the platelet receptor CD 62P [14,15], possibly promoting a pro-inflammatory monocyte phenotype with an increased excretion of cytokines such as TNF-a, IL-1β and IL-8 [16] and a phenotypic change towards *intermediate* subsets by increasing CD 16$^+$ expression [17]. Monocyte-platelet aggregates (MPA) are upregulated after AMI [18] and correlate with adverse cardiac events [19] and endothelium dependent coronary vasomotor dysfunction [20]. The routine administration of dual antiplatelet therapy (DAPT) with ASA and P2Y$_{12}$ inhibitors – aimed at the prevention of ischemic complications – also reduces MPA formation, expression of proinflammatory cytokines and C-reactive protein (CRP) [21–23]. Administration of the P2Y$_{12}$ inhibitors clopidogrel leads to a more profound reduction of CRP, TNF-α, and MPA-formation compared to ASA alone [24]. Even more potent inhibition of P2Y$_{12}$ by prasugrel may further reduce proinflammatory effects of platelets [25].

In this study, we retrospectively analyzed the influence of platelets on the monocytic inflammatory response, local myocardial inflammation as assessed by ^{18}F-FDG-PET/MRI, and left ventricular function in the course of 6 months after AMI. In this context, we also evaluated the potential influence of antiplatelet therapy on MPA formation and myocardial inflammation.

2. Methods

2.1. Patient population

This is a retrospective analysis of data from 58 STEMI patients admitted to the University Hospital rechts der Isar and the German Heart Centre, Munich, between May 2013 and April 2015. 29 patients were enrolled in a previously published imaging study, evaluating PET/MRI for the visualization of myocardial inflammation [11]. Patients from this population ("core population") were included in the current analysis if MPA measurements were available. To evaluate some findings of the analysis in an extended population, additional 29 patients with STEMI with MPA data available, who did not undergo PET/MRI (see Suppl. Table 1), were included. All patients provided informed consent. The study was approved by the local ethics committee and was performed in agreement with the Declaration of Helsinki. No patients received GPIIb/IIIa antagonists or had an indication for anticoagulant therapy.

2.2. Blood sampling

Blood samples for the analyses of monocyte subpopulations and monocyte-platelet aggregates were taken at the presumptive peak within the first 72 h after MI during hospitalization (51 patients within the first 48 h, 7 patients within 72 h), according to the initial study protocol and corresponding to a time window for which roughly constant monocyte levels have been demonstrated in patients and mice [5,18].

2.3. Flow cytometry analyses

Flow cytometries were performed on a Cytomics FC 500 flow cytometer and analyzed with Kaluza 1.2 (both Beckman Coulter, CA, USA). Whole blood was lysed, stained with anti-human monoclonal fluorochrome-conjugated antibodies, and immediately analyzed. Gating was performed similar to previously described strategies [2,9,26,27]. CD41 was used to identify monocyte-platelet aggregates as previously described [18,23,28]. A detailed description of the gating strategy is provided in Suppl. Fig. 6. Monocyte subpopulation counts were extrapolated from differential blood counts and relative fractions in flow cytometry. MPAs were defined as monocytes with positivity for the platelet marker CD41.

2.4. Flow cytometry reagents

Versa Lyse, anti-CD45-ECD (clone J.33), anti-CD14-PC5 (Clone RMO52), anti-CD16-PC7 (Clone 3G8), anti-CD41-PE (Clone P2) Cytomics FC100 flow cytometer, Kaluza software (all Beckman Coulter, CA, USA).

2.5. PET/MR imaging

Simultaneous PET/MRI imaging was performed as described previously [11]. In brief, to determine the ^{18}F-FDG uptake volume, a region grow algorithm with a threshold of 50% of the maximal uptake was performed (Syngo MMWP [workstation], Syngo TrueD [software]; Siemens Medical Solutions). From this volume of interest (VOI), the SUV$_{mean}$ was derived and normalized to the lean body mass [29]. Detailed information on image acquisition and analysis can be found in the Methods section and supplement of the previous publication [11].

2.6. Assessment of ΔEF

The change of ejection fraction between baseline and follow-up was assessed in patients with serial imaging in the same imaging modality, preferably cardiac MRI (28 patients). In patients without follow-up MRI, initial and follow up LV-EF was calculated from serial biplane echocardiographic images using the Simpson biplane-method ($n = 2$) or serial LV-ventriculography using the single-plane area-length technique ($n = 9$), resulting in 39/58 patients with ΔEF available. EF analyses were performed in a blinded fashion.

2.7. Statistics

Results are shown as mean ± standard deviation unless indicated otherwise. For comparison of continuous variables, Student's *t*-test was used. For comparison of 3 groups, one-way ANOVA with Scheffé post hoc analysis was used. In datasets not meeting the criteria for ANOVA, Kruskal-Wallis test and Conover post hoc analysis was used. For comparison of categorical variables, chi-square test was used. Samples were tested for normal distribution with the Kolmogorov-Smirnov test. Association between continuous variables was investigated using Pearson correlation coefficient. For simplification, *p*-values smaller than 0.05 were termed significant in the text. However, it should be appreciated that due to the retrospective nature and the conduct of multiple comparisons, these results have no confirmative character and should be viewed as hypothesis generating. For statistical analyses, MedCalc 15.8 (Ostend, Belgium) and Excel 2017 (Microsoft Inc., Redmond, USA) were used.

3. Results

3.1. Patient characteristics

Data from 29 patients with STEMI with available PET/MRI and data on monocyte subpopulations and MPAs were retrospectively analyzed. Data from 29 additional patients with MPA data but without PET/MRI were included in an extended analysis (see Suppl. Table 1). Patient characteristics of the two groups are presented in Table 1.

3.2. CD16$^+$-MPA percentage is associated with myocardial ^{18}F-FDG uptake

Patients after AMI with above median MPA percentages (% of monocytes with platelets on surface) of CD14^{high}CD16$^+$ and CD14^{low}CD16$^+$ monocytes showed significantly more local ^{18}F-FDG uptake (SUV$_{mean}$) in the infarcted myocardium than patients with MPA percentage of these monocyte subpopulations below the median ($p < 0.05$, Fig. 1 middle and right panel, $n = 29$), while MPA percentage of CD14^{high}CD16$^-$ monocytes was not associated with ^{18}F-FDG uptake (Fig. 1, left panel). Absolute MPA counts had no influence on myocardial ^{18}F-FDG uptake (Suppl. Fig. 1).

3.3. High MPA percentages are associated with adverse functional outcome

Based on the association of CD16$^+$-MPA percentage with myocardial ^{18}F-FDG uptake and our previously reported finding of an association between ^{18}F-FDG uptake and deteriorating systolic function [11], we assumed a potential relationship between MPA percentages and functional outcome. Indeed, in the core population, above median MPA percentages were associated with deteriorating systolic LV function

Table 1
Patient characteristics of the core population and the extended analysis population.

Characteristics	Core population ($n = 29$)	Extended analysis population ($n = 58$)	p-Value
Male	21 (72%)	48 (83%)	$p = 0.34$
Age	58 ± 13	59 ± 12	$p = 0.6$
MI localisation-no. (%)			
Anterior	18 (62%)	30 (52%)	$p = 0.36$
Inferior	7 (24%)	20 (34%)	$p = 0.33$
Lateral	4 (14%)	8 (14%)	$p = 0.95$
TIMI III post PCI	25 (86%)	53 (91%)	$p = 0.46$
TIMI II post PCI	4 (14%)	5 (9%)	$p = 0.46$
Cardiovascular related history-no. (%)			
Smoker (current)	10 (34%)	21 (36%)	$p = 0.87$
Smoker (former)	7 (24%)	12 (21%)	$p = 0.71$
Hypertension	15 (52%)	35 (60%)	$p = 0.44$
Diabetes mellitus	4 (14%)	14 (24%)	$p = 0.26$
Dyslipidemia	7 (24%)	20 (34%)	$p = 0.37$
$P2Y_{12}$ inhibitors-no. (%)			
Clopidogrel	12 (41%)	14 (24%)	$p = 0.1$
Prasugrel	10 (34%)	26 (45%)	$p = 0.36$
Ticagrelor	7 (24%)	18 (31%)	$p = 0.56$
LVEF, % baseline	47 ± 10	47 ± 9	$p = 0.97$
CK max., U/l	2399 ± 2181	2327 ± 2102	$p = 0.89$
TnT max., ng/ml	3,7 ± 5,1	3,8 ± 4,4	$p = 0.45$
Leukocyte count max.	12,4 ± 3,1	12,5 ± 4,2	$p = 0.97$
CRP max.	2,5 ± 3,2	1,6 ± 2,7	$p = 0.21$

after 6 months compared to patients below the median for all monocyte subpopulations ($p < 0.01$, Fig. 2, $n = 25$). This was also observed for MPA percentage of $CD14^{high}$ monocyte populations in the extended analysis (Suppl. Fig. 2 A, $n = 39$). In contrast, absolute MPA counts were not associated with adverse remodeling (Suppl. Fig. 2 B).

3.4. Influence of $P2Y_{12}$ inhibitor choice on MPA percentage and myocardial ^{18}F-FDG uptake

Patients received dual antiplatelet therapy, usually with ASA and either ticagrelor or prasugrel. However, 12 patients in the core population (14 in the extended population) received clopidogrel by decision of the interventionalist, allowing us to compare MPA counts and percentages in STEMI patients treated with three different $P2Y_{12}$ inhibitors. No significant differences regarding age and infarct size were observed between patients treated with different $P2Y_{12}$ inhibitors. In the core population, the MPA percentages of $CD14^{high}CD16^{-}$ ($p < 0.01$, Fig. 3 A, left panel) and $CD14^{high}CD16^{+}$ monocytes ($p < 0.01$, Fig. 3 A, middle panel) were significantly lower in patients receiving ticagrelor compared to patients receiving clopidogrel. In the extended population, MPA percentages were significantly lower in all monocyte subpopulations in patients receiving Ticagrelor in comparison to patients receiving prasugrel and clopidogrel ($p < 0.05$, Suppl. Fig. 3 A), despite similar degree of platelet inhibition in patients receiving prasugrel vs. patients receiving ticagrelor ($p < 0.05$; Suppl. Fig. 3 C). Drug choice did not significantly affect absolute MPA counts (Suppl. Fig. 3 B). With regard to myocardial inflammation, patients receiving ticagrelor ($n = 7$) and prasugrel ($n = 10$) displayed a trend towards a reduced local ^{18}F-FDG uptake in the infarct area compared to patients treated with clopidogrel ($n = 12$; Fig. 3 B).

3.5. MPA counts are related to monocyte count and subtype

MPA percentages of any monocyte subpopulations did not correlate with infarct size measured by LGE (Suppl. Fig. 4 A). However, the absolute MPA count of $CD14^{high}CD16^{+}$ monocytes weakly correlated with infarct size (LGE extent; $r = 0.45$, $p < 0.05$, Suppl. Fig. 4 B middle panel), paralleling the association of monocyte counts and infarct size [11]. Consequently, total MPA counts of $CD14^{high}CD16^{-}$ ($r = 0.76$, $p < 0.01$ Suppl. Fig. 5 A left panel) and $CD14^{high}CD16^{+}$ monocytes ($r = 0.63$, $p < 0.01$, Suppl. Fig. 5 A middle panel) correlated with total counts of the respective monocyte subpopulations. Conversely, total thrombocyte counts did not affect the relative MPA percentage and total MPA counts (Suppl. Fig. 5 B and C). In the extended analysis, average MPA percentage differed among monocyte subpopulations and overall appeared higher in $CD14^{high}$ monocytes compared to $CD14^{low}$ monocytes (Suppl. Fig. 5 D, $p < 0.05$).

4. Discussion

Despite the advances in the treatment of AMI, adverse remodeling and heart failure is a persisting clinical problem. The immune system

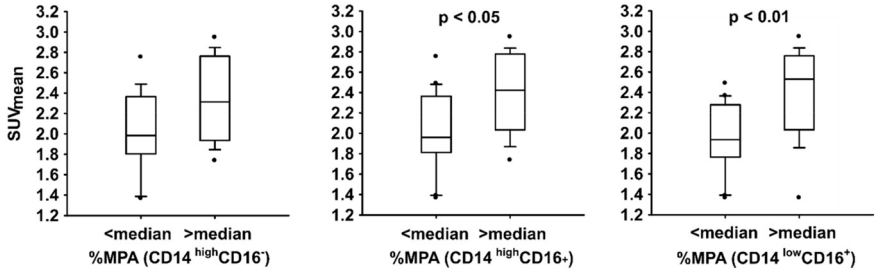

Fig. 1. MPA percentages of $CD16^{+}$ monocytes are associated with ^{18}F-FDG uptake in the myocardium. The average local ^{18}F-FDG uptake (SUV_{mean}) in the infarcted myocardium is depicted in relation to the MPA percentages of $CD14^{high}CD16^{-}$ (left panel), $CD14^{high}$ $CD16^{+}$ (middle panel), and $CD14^{low}$ $CD16^{+}$ (right panel) monocytes. Patients with MPA percentages of $CD16^{+}$ monocytes above the median showed stronger ^{18}F-FDG uptake than patients with MPA percentages of these monocyte subsets below the median. Data from the core population ($n = 29$) are displayed as box and whisker plots. Boxes display 25–75%, error bars display 5th and 95th percentile for each dataset.

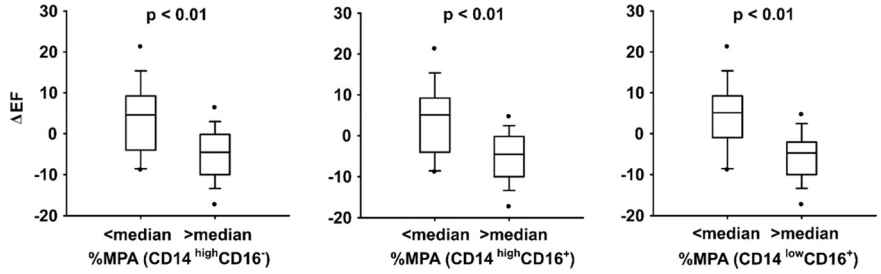

Fig. 2. High MPA percentages are associated with deteriorating systolic LV function after 6 months. ΔLV-EF after 6 months in patients below and above the median of MPA percentage for $CD14^{high}CD16^{-}$ (left panel), $CD14^{high}CD16^{+}$ (middle panel), and $CD14^{low}CD16^{+}$ (right panel) monocytes is shown, demonstrating improvement of systolic function in patients with MPA percentages below the median compared to patients with MPA percentages above the median for all analyzed monocyte subtypes. Data from patients in the core population with follow-up MRI ($n = 25$) are displayed as box and whisker plots. Boxes display 25–75%, error bars display 5th and 95th percentile for each dataset.

has gained increasing interest as the main orchestrator of endogenous cardiac repair and scar formation. In this context, the right degree of inflammation is necessary for adequate post-infarct repair. This process is tightly regulated, with monocyte subpopulations and macrophages being at the center of the innate immune response. Different immune-modulating approaches adjusting the inflammatory response post MI in patients to prevent adverse remodeling have shown divergent results to date [30], suggesting that a more precise identification of individuals at risk for a harmful immune response may be needed to define potential therapy responders. Assessment of the myocardial inflammation with ^{18}F-FDG-PET/MRI may be a tool to identify these patients, particularly as this biosignal correlated with the functional outcome after 6 months in our previously published study [11], supporting the potential clinical relevance of this novel imaging strategy. Although the cellular substrate of the ^{18}F-FDG signal in this context has been identified as monocytes/macrophages in a rodent study [10], the lack of correlation between the intensity of local ^{18}F-FDG-PET and standard peripheral blood markers of inflammation in our previous study raised the question, which mechanisms regulate the local intensity of inflammation in the myocardium [11]. Examining different factors modulating the monocytic transmigration to the ischemically compromised myocardium, we also focused on platelets, as monocytes-platelet crosstalk is increased up to 80% following AMI [18] and believed to be a crucial pathophysiological mechanism linking thrombosis and inflammation [31].

Our present analysis indicates that the degree of monocyte-platelet aggregation may directly translate into the intensity of myocardial inflammation. In detail, the average intensity of myocardial inflammation in the infarcted myocardium assessed as SUV_{mean} was significantly higher in patients with MPA percentage of $CD16^{+}$ monocytes above the median. Interestingly, absolute peripheral monocyte (as shown previously) [11] and MPA counts were associated with infarction size and thus the infiltrated myocardial volume, but did not reflect the average local intensity of the ^{18}F-FDG-PET signal in our patient cohort. In

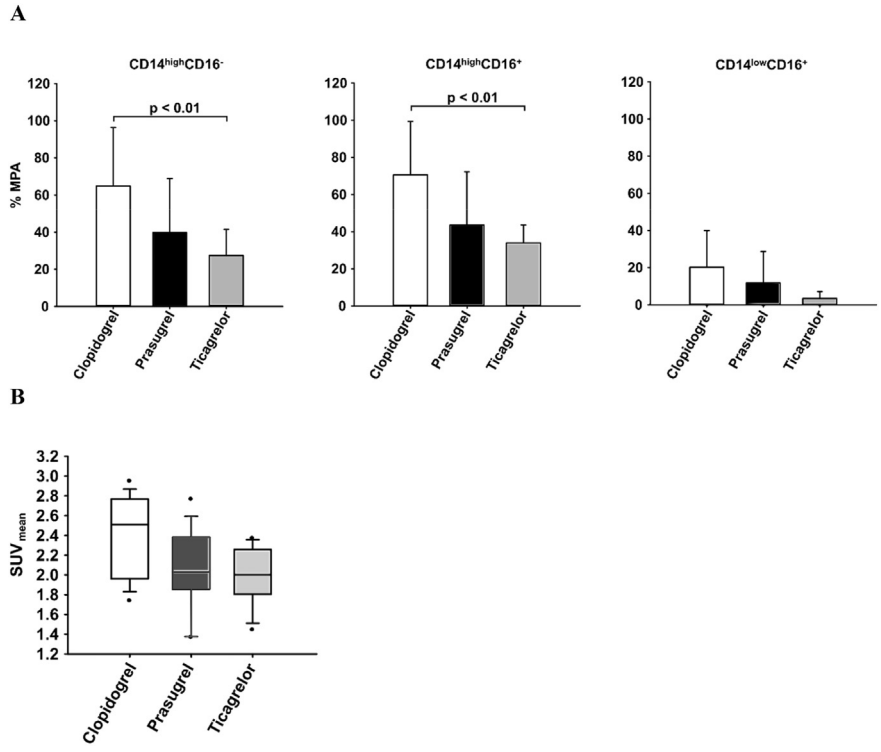

Fig. 3. Influence of $P2Y_{12}$ inhibitor choice on MPA percentage and ^{18}F-FDG uptake in myocardium. A: The influence of $P2Y_{12}$ inhibitors on the MPA percentage of $CD14^{high}CD16^{-}$ (left), $CD14^{high}CD16^{+}$ (middle), and $CD14^{low}CD16^{+}$ (right) monocyte subpopulations is shown. In each panel, data from patients receiving the different $P2Y_{12}$ inhibitors clopidogrel ($n = 12$), prasugrel ($n = 10$) and ticagrelor ($n = 7$) are compared, showing significantly lower MPA percentages in patients treated with ticagrelor compared with clopidogrel. Data (core population) are displayed as box and whisker plots. Boxes display 25–75%, error bars display 5th and 95th percentile for each dataset. B: ^{18}F-FDG uptake (SUV_{mean}) in the post-ischemic myocardium in relation to $P2Y_{12}$ inhibiting drug is shown, comparing patients from the core population receiving clopidogrel ($n = 12$), prasugrel ($n = 10$) and ticagrelor ($n = 7$). Data is shown as mean ± SD.

contrast, MPA percentages of monocyte subpopulations were associated with average ^{18}F-FDG-PET signal intensity (SUV_{mean}) in the infarcted area but rather independent of infarct size. Taken together, the current analyses suggest that the infarction size may be a factor determining the overall quantity of monocyte release from spleen and bone marrow via nervous and humoral signals, thereby assuring an adequately sized immune response to repair damaged tissue. In contrast, the local intensity or "concentration" of inflammation in the infarct (represented by SUV_{mean}), i.e. the amount of local leukocyte extravasation, appears rather independent of infarction size and regulated by other factors. Here we present evidence that monocyte-platelet aggregation may be one such factor influencing monocyte extravasation. In line with this hypothesis, MPA formation has been shown to directly upregulate receptors for adhesion molecules in monocytes and endothelial cells and facilitates monocyte migration [32–35], while platelet depletion in an in vitro model abolished monocyte recruitment over Endothelial Cells (EC) [34].

Our previous PET/MRI study revealed a relationship of ^{18}F-FDG uptake after 5 days and ΔLV-EF after 6 months [11], stressing the clinical significance of the underlying pathophysiology. In the current analysis, we were able to show that patients with high MPA percentage experienced deterioration of their systolic LV function over the following 6 months while patients with low relative MPA percentage had improved systolic function, supporting the pathophysiologic relevance of these results. In line with a previously published study demonstrating that levels of $CD14^{high}CD16^{+}$-MPAs are inversely associated with ΔLV-EF in patients 6 weeks after MI [19], our results suggest a potential impact of monocyte platelet interactions on intermediate and long-term infarct healing and remodeling processes. This particularly seems to be relevant for MPA of $CD14^{high}$ $CD16^{+}$ monocytes, the monocyte subset that has been ascribed a key role in modulating of inflammation [36,37].

Finally, we examined a potential influence of antiplatelet therapy on monocyte-platelet interaction and inflammation. Interestingly, treatment with ticagrelor was associated with a significantly lower MPA percentage compared to treatment with clopidogrel (core population) and clopidogrel and prasugrel (extended population). Besides the known positive effects of antiplatelet therapy with clopidogrel and ASA on endothelial function [38] and a dose dependent effect on platelet activations status [39], the more potent DAPT therapy with prasugrel showed significantly decreased MPA formation in patients with stable CAD [25] – resulting in decreased release of pro-inflammatory cytokines from a-granules [40]. Nevertheless, the difference in MPA formation between ticagrelor and prasugrel cannot be explained by $P2Y_{12}$ inhibiting potency, which comparable for both compounds [41]. Interestingly, immune modulating properties of ticagrelor have been suggested by a substudy of the PLATO study [42]. A possible mechanism for these effects may be found in drug actions of ticagrelor besides $P2Y_{12}$ inhibition. Ticagrelor leads to increased levels of adenosine in patients with ACS [43] and adenosine in higher concentrations acts anti-inflammatory via downregulation of pro-inflammatory cytokines such as IL-6 and TNFα [44,45]. Alternatively, the differing pharmacodynamics and pharmacokinetics of prasugrel and ticagrelor might partially explain these results. Prasugrel is a prodrug with a briefly circulating active metabolite that irreversibly binds platelets, while ticagrelor is a reversible inhibitor with a comparably long half-life. Due to platelet turnover and daily mobilization of immature platelets from the bone marrow, this difference leads to reduced numbers of platelets being inhibited at the end of the dosing interval with prasugrel compared to ticagrelor [46]. Taken together, with all caution in the interpretation of these findings, they support the hypothesis that antiplatelet therapy with ticagrelor might decrease the relative MPA percentage of monocytes, particularly of the $CD14^{high}$ $CD16^{+}$ subset. This in turn may explain the trend towards a reduced ^{18}F-FDG uptake at the site of ischemia.

In summary, our current analysis indicates that monocyte-platelet interactions after AMI are associated with myocardial tissue inflammation in patients and may thereby influence functional outcome after AMI. Furthermore, our observations suggest that the choice of standard antiplatelet therapy after AMI may directly influence myocardial inflammation. Particularly a therapy with ticagrelor was associated with low MPA percentages, which may not entirely be explained by the drug's $P2Y_{12}$ inhibitory potency. In line with this hypothesis, a beneficial effect of ticagrelor on cardiac post-MI remodeling has recently been suggested by a porcine AMI study [47]. Although the concept of platelet-monocyte crosstalk in inflammation is not new, our results hint towards a clinical significance that is not being appreciated by current infarction therapy. In perspective, the choice of antiplatelet therapy may influence the patient's individual inflammatory risk. Ongoing clinical trials evaluating $P2Y_{12}$ inhibitor therapy, as the ISAR-REACT 5 trial [48], comparing Ticagrelor and Prasugrel in patients with acute coronary syndromes, may give additional insight into this question.

Our study has several limitations, most importantly its observational, retrospective nature and small sample size. Furthermore, it must be appreciated that neither the causality between MPAs and myocardial inflammation, nor the direction of potential causality can be concluded from our study. Increased MPA levels may as well be an event downstream of exaggerated inflammatory response. Moreover, the reasoning for antiplatelet drug choice was not known for all cases and may represent a source of considerable bias. Another limitation is the linkage between peripheral blood cell numbers and tissue inflammation, as both follow distinct kinetics after AMI. For our study, both measures were taken around the time of the assumed peak of their kinetics - monocyte and MPA assessment were carried out during the first 72 h after AMI, when peripheral monocyte counts peak (driven by the mobilization of the $CD14^{high}$ monocytes) [18,19], and ^{18}F-FDG-PET was performed at its presumed peak at day 5 after AMI, which was extrapolated from preclinical data [11]. Taken together, our results must be considered as hypothesis-generating. Yet, the observations from our analysis are appealing, as they fit pathophysiologic principles linking thrombocyte biology with inflammation. Independent prospective studies will be needed to evaluate the relevance of these findings.

Author contributions

H.K., R.J.D., C.R., S.G.N., D.S., T.I., M.S., and K.-L.L. designed the study, C.R., S.G.N., K.P.K. and M.S. established the imaging strategy and performed imaging analyses, H.K., K.G., F.H. and R.J.D. established the flow cytometry strategy and performed flow cytometry analyses, H.K., R.J.D., D.S., T.I., A.K., and K.-L.L. recruited patients, H.K., C.R., F.H., and R.J.D. analyzed data, H.K. and R.J.D. wrote the manuscript, and A.G., H.S., C.K., T.I., M.S., and K.-L.L. provided expertise and revised the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.04.009.

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