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Increased expression of TF on monocytes, but decreased numbers of TF-bearing microparticles in blood from patients with acute myocardial infarction - a pilot study

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Introduction

Occlusive thrombus formation after disruption of an atherosclerotic plaque is thought to be the cause of acute myocardial infarction (AMI). Tissue factor (TF) is regarded as a key regulator of this process (1). TF is a membrane protein that triggers blood coagulation. In complex with factor VIIa it catalyzes the activation of factors IX and X, leading to thrombin and subsequent fibrin formation. While tissue factor (TF), which is present in the damaged vessel wall, is necessary for haemostasis, various studies signify that circulating TF plays a central role in pathological thrombus growth (reviewed in (1). Activated monocytes and circulating microparticles (MP), probably derived from activated monocytes, are a potential source of this 'blood-borne' TF. According to the current concept of cell-based coagulation, circulating MPs and monocytes interact with activated platelets at the site of plaque rupture and offer a mechanism for TF delivery (1, 2).

Activated platelets that bind to circulating monocytes form monocyte-platelet aggregates (MPA). It was revealed that these complexes show increased adhesive capacities but also facilitate recruitment of noncomplexed monocytes at the site of injury (3).

Previous studies have shown that TF expression on monocytes and monocyte-platelet aggregates (MPA) are increased in acute coronary syndromes (4, 5). It was also reported that patients with acute coronary syndromes had increased levels of circulating MPs of platelet, endothelial and leukocyte origin (6). In addition, Seljeflot et al followed patients after MI for 4 years and showed that patients with reinfarction or stroke had significantly higher plasma tissue factor levels as compared to those who did not (7). We therefore hypothesize that measurement of circulating TF in AMI could provide clinically relevant diagnostic and prognostic information.

In this study flow-cytometry was used to determine 1) the expression of TF on monocytes, 2) monocyte-platelet aggregates (MPA) and 3) TF positive MPs in blood of patients with AMI and healthy controls. We hypothesize that these assays show sensitivity and specificity for AMI. To the best of our knowledge this is the first study that measures specifically circulating TF-bearing MPs in patients with AMI using flow-cytometry.

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Methods

Blood was collected in two vacutainer tubes (Becton Dickinson) containing respectively trisodium citrate and EDTA from 12 patients presenting with AMI (troponin T>0.1 µg/L) at our emergency department and 23 healthy controls.

Monocytes and microparticles were measured using a FACSCanto flow cytometer with FacsDiva Software (Becton Dickinson). Triggering was set at forward scatter (FSC).

The EDTA blood was used to separate mononuclear cells from whole blood using a commercially available cell preparation tube (CPT™, Becton Dickinson). Mononuclear cells were stained with fluorescently labelled antibodies against monocytes (a-CD14-PE, Becton Dickinson), platelets (a-CD41-PerCP, Becton Dickinson) and TF (a-TF-FITC, Sanquin). The population of monocytes was identified by CD14-labeling. The monocytes were selected by a gate. Using bivariate plots, we then determined the percentage of cells expressing TF and the percentage of cells expressing CD41, representing MPA.

Citrated blood was centrifuged at 3000 g for 10 minutes. This centrifugation step was optimised to separate MP from blood cells and platelets. MPs were measured as described previously (8). In the platelet free plasma TF-bearing MPs were stained with a TF-FITC. To determine MP concentration flow count beads (Beckman Coulter) were added in a 1:10 dilution to the sample. Flow count beads (10 um) fall in a size gate different from MP. Estimates of TF positive MP concentration were calculated with the following equation:

$$\text{TF positive MP}_{conc.} = \text{bead}_{conc.} \times (\text{TF positive MP}_{count} / \text{bead}_{count})$$

In both the monocyte and the microparticle assay fluorescence thresholds for monoclonal antibodies were set in terms of binding of isotype-matched control antibodies (IgG_1).

The data were expressed as mean ± standard deviation. To determine the statistical significance of differences, probability values were obtained with a non-parametric test (Mann Whitney U) for two unpaired variables. Differences were considered significant at $p<0.05$.

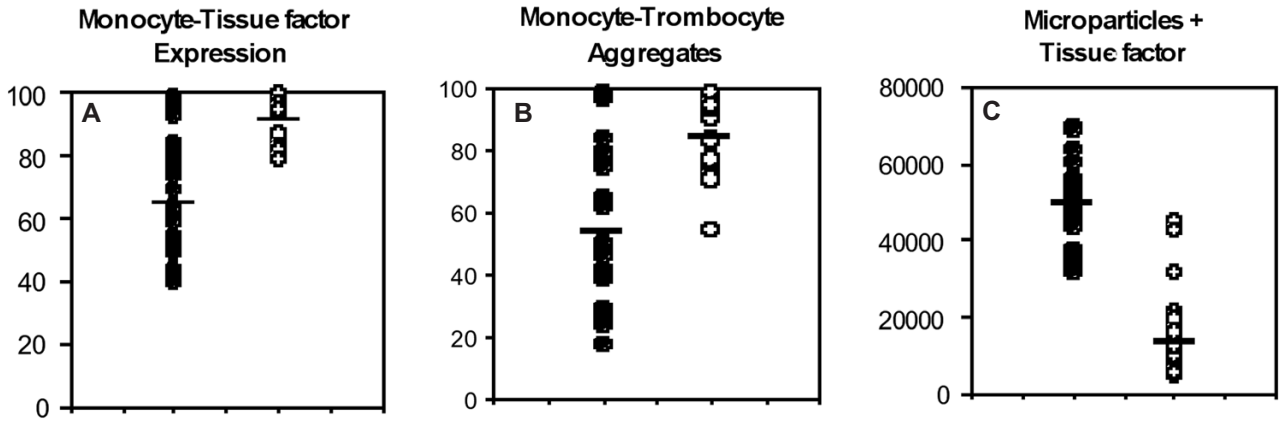

Figure 1. Procoagulant characteristics of monocytes and microparticles in patients with AMI and healthy controls. Shown are (A) the percentage of monocytes that express TF, (B) the percentage of monocytes in complex with thrombocytes and (C) the concentration of microparticles that express TF at their membrane surface in peripheral blood of control subjectes (●) and patients with AMI (○).

Results

As depicted in figure 1, TF expression on monocytes in AMI patients (91 ± 7%) was significantly (p=0.003) elevated with respect to controls (65 ± 25%). In addition, the percentage of MPA was significantly increased in AMI with respect to controls (84 ± 14% versus 58 ± 27%; p=0.007).

Receiver operating characteristic (ROC) curves plot sensitivity against false positive rate (1-specificity) for all possible cut-off values of a diagnostic test. Assay performance is assessed as the area under this curve (AUC). The ROC curve for the monocyte TF and MPA assay displayed both sensitivity and specificity for AMI, the AUC being 0.81 and 0.78, respectively. Using a threshold value of 76% for the monocyte-TF assay, the sensitivity for AMI was 100% and the specificity 65%. When using a threshold value of 51% for the MPA assay the sensitivity for AMI was 100% and the specificity 61%.

To our surprise, the numbers of circulating MPs with TF on their membrane were not elevated in AMI. Moreover, figure 1 shows that there is a significant decrease of TF-bearing MPs in peripheral blood of AMI patients with respect to controls (24 ± 30versus 50 ± 16 MPs/nl; p=0.0001).

Discussion

In accordance with current literature, we confirmed that TF expression on monocytes and circulating MPA were increased in patients with AMI (4, 5). We further demonstrated that both parameters showed diagnostic sensitivity and specificity for AMI. Whether these two parameters give prognostic information for future events or survival is subject for further studies.

An important finding of this study was the decreased number of TF-bearing MPs in peripheral blood of patients with AMI with respect to controls. Our findings are in line with a recent study of Maly et al, who demonstrated that TF activity and MP count in platelet free plasma of patients with acute coronary syndromes were reduced (9). We realize that because of the limited numbers of patients and controls in our study, these results should be interpreted with caution. Yet, this finding is in full accordance with the current concept of cell-based coagulation (1). The model assumes that active TF that is bound to MPs is recruited from the circulating blood by binding to a surface of activated cells, as in a developing thrombus. We therefore speculate that the depletion of procoagulant MPs in the peripheral blood might be caused by adhesion to the coronary thrombus. Indeed, Morel et al demonstrated recently in patients with AMI that MP numbers were considerably higher within the occluded artery than in peripheral blood samples (10).

Overall, this study shows that procoagulant characteristics of circulating monocytes are increased in peripheral blood of patients with AMI. Flow cytometric analysis of monocyte TF expression and MPA has diagnostic sensitivity and specificity for AMI. We further conclude that numbers of TF-bearing MPs in peripheral blood are decreased in AMI, probably due to recruitment at the site of injury. Whether the procoagulant characteristics of monocytes and the number of TF-bearing MPs after AMI give prognostic information for future events needs to be studied.

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Resultaten van reflecterend testen bij 1e-lijnslaboratoriumuitslagen

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Inleiding

Laboratoriumdiagnostiek in de eerste lijn wordt veelal toegepast om patiënten met bekende aandoeningen te monitoren. Indien toegepast voor diagnostiek, wordt laboratoriumonderzoek vaker gebruikt om ziekten uit te sluiten dan aan te tonen. Vaak is de differentiele diagnose echter niet duidelijk en wordt er screenend laboratoriumonderzoek ingezet. In een deel van de gevallen worden in de laboratoriumuitslagen afwijkingen gevonden die duiden op bepaalde pathologie. Voor de herkenning en interpretatie hiervan kan het laboratorium, als vorm van extra service, behulpzaam zijn. Hiervoor is het noodzakelijk dat een laboratoriumspecialist de uitslagen beoordeelt, en zo nodig aanvullende testen toevoegt om de diagnostiek te completeren. Er wordt bijvoorbeeld een licht verhoogd (ongeconjugeerd) bilirubine gevonden. Het toevoegen van bijvoorbeeld een haptoglobinebepaling zal hier richting geven aan de diagnostiek door het uitsluiten van hemolyse. De laboratoriumspecialist kan de huisarts wijzen op de mogelijkheid van het syndroom van Gilbert, een onschuldige, aangeboren verhoging van het ongeconjugeerde bilirubine. Het ligt in dit soort gevallen voor de hand dat het laboratorium zelf het initiatief neemt om deze aanvullende testen uit te voeren. Bovendien is een bloedmonster beschikbaar, waarmee in de meeste gevallen de aanvullende testen kunnen worden uitgevoerd. Deze procedure is de patiënt tot voordeel omdat de diagnostiek sneller afgerond kan zijn. De patiënt blijft bovendien een tweede bloedafname bespaard. Voor deze procedure, waarbij de laboratoriumspecialist beoordeelt of aanvullende testen nodig zijn, is de term ‘reflective testing’ (reflecterend testen) ingevoerd (1). Er wordt gerichte diagnostiek uitgevoerd bij patiënten met voldoende pre-testwaarschijnlijkheid op een aandoening. Deze service verschilt van ‘reflextesten’, waarbij een van tevoren vastgesteld testprotocol automatisch wordt doorlopen. Reflecterend testen biedt de mogelijkheid om richtlijnen te effectueren en vraagt een proactieve houding van het laboratorium. In de laboratoria van het Verenigd Koninkrijk wordt reflecterend testen gezien als integraal onderdeel van de dienstverlening (2).

Nieuwe technieken van dataverwerking maken deze vorm van werken voor het laboratorium mogelijk. De ontwikkeling van het elektronisch patiëntendossier maakt het mogelijk een goede indruk te krijgen van de klinische situatie van de patiënt. Bovendien maakt toegepaste software het mogelijk om sterk afwijkende uitslagen en afwijkende patronen automatisch te herkennen (3). De afweging om testen wel of niet toe te voegen is geen eenvoudig proces. Hiervoor is vakinhoudelijke, medische kennis noodzakelijk om de wenselijkheid van aanvullende diagnostiek in te schatten en de juiste testen te selecteren.

Het laboratorium van het Atrium Medisch Centrum te Heerlen is in juni 2006 gestart met reflecterend testen bij aanvragen van huisartsen. Vergoeding van toegevoegde testen vindt plaats onder de naam van de betreffende huisarts. In eerdere studies hebben wij aangetoond dat de huisartsen in oostelijk Zuid-Limburg het op prijs stellen dat het laboratorium het initiatief neemt om testen en commentaren toe te voegen (4, 5). Bovendien hebben wij aangetoond dat onze werkwijze vrijwel altijd als zinvol werd ervaren. Volgens de betreffende huisartsen werd het patiëntbeleid in meer dan de helft van de gevallen op een positieve manier beïnvloed (6). Toevoegen van ons commentaar leidde vaak tot aanpassing van medicatie bij bijvoorbeeld schildklieraandoeningen, aanvullende diagnostiek zoals een

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