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Macrophage, eosinophil, and mast cell extracellular traps (METs, EETs and MCETs) participate in coronary thrombus evolution after acute myocardial infarction

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Topic: myocardial infarction, acute, eosinophils, macrophages, mast cells, thrombus, extracellular traps

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On behalf of: Cardiovascular Pathology

Background: Extracellular traps generated by neutrophils (NETs) play important roles in the formation and propagation of the atherothrombotic mass following coronary plaque disruption, and are thus presumed to contribute to the ensuing onset of acute myocardial infarction. However, other cells such as macrophages, eosinophils and mast cells, may also capable of releasing extracellular traps, which are called as METs, EETs and MCETs, respectively. The generation of these extracellular traps also marks a distinct form of cell death namely etosis.

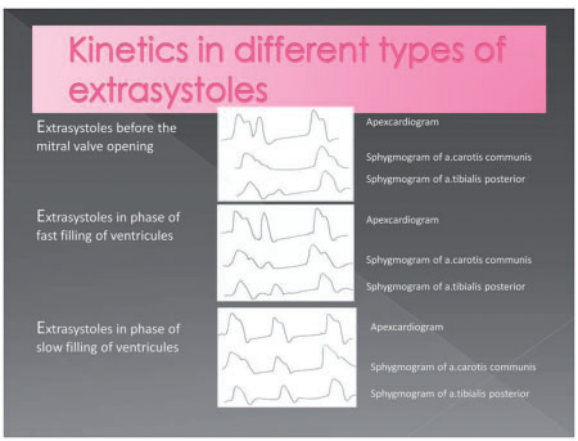
Purpose: The aim of this study was to investigate the formation of NETs, METs, EETs and MCETs in human coronary thrombectomy specimens of myocardial infarction (AMI) patients, in relation to the age of the thrombus.

Method: Thrombectomy specimens obtained from 48 AMI patients were available in paraffin sections for this study. Using HE-stains, they were classified as either 25 fresh (<1 day old, intact erythrocytes and granulocytes), 25 lytic (1-5 days old, lytic changes) or 19 organised (>5 days, fibrocellular ingrowth) thrombi. Immunohistochemistry was performed to identify neutrophils (MPO), macrophages (CD68), eosinophils (EMBP) and mast cells (tryptase). NETs, METs, EETs and MCETs were visualised in double-immunostains using the cell specific antibodies in combination with anti-citrullinated histone-3 (CitH3) antibody. Single and double- immunostained cells were counted as number/mm^2 and calculated as the average numbers/mm^2 for each thrombus category.

Results: NETs, METs, EETs and MCETs were present in all different thrombus age. Fresh thrombi contained more NETs (167/mm^2), followed by METs (43/mm^2), EETs (10/mm^2) and MCETs (4/mm^2)($p<0.05$); lytic thrombi had more NETs (120/mm^2) and METs (101/mm^2) compared to EETs (2/mm^2) and MCETs (2/mm^2)($p<0.05$); and organised thrombi contained more METs (37/mm^2), followed by NETs (25/mm^2), MCETs (8/mm^2) and EETs (2/mm^2) ($p<0.05$).

Conclusion: Not only neutrophils, but also macrophages, eosinophils and mast cells are able to generate extracellular traps and undergo etosis during the evolution of coronary thrombosis. Their relative participation depends on the organisation stage of the thrombus.

parameters of heart biomechanics and main arteries kinetics (carotid, radial, ulnaris, posterior tibia, arch of foot arteries), which characterized speed, acceleration, capacity and work in each phase of heart cycle in systole and diastole, and also the periods of dominance of outflow over inflow. For that we used by doppler-ultrasound and sphygmography. We analyzed the peak speed direct blood flow, blood flow volume. We valued the contribute to the circulation of the premature contraction and first post-extrasystolic contraction. The volume of cardiac output and transmitral blood flow were measured by echocardiography.
We identified the moment of extrasysyoles' appearance by using apex-cardiography and ECG.
We classified extrasystoles in accordance to the moment of their appearance in cardio cycle. We have identified:
Extrasysoles before the mitral valve opening.
Extrasystoles in rapid ventricular filling phase before the transmitral blood flow peak.
Extrasystoles in rapid ventricular filling phase after the transmitral blood flow peak.
Extrasystoles in slow ventricular filling phase.
Results: and conclusion. The main role in hemodynamic and kinetics changes play the time of extrasystole appearance in cardio cycle and the ability of the first post-extrasystolic contruction to reestablish an adequate resulting blood flow. If there's a patient with multifocal atherosclerosis in main arteries the sharp increase of hemodynamic and kinetic parameters of arteries increase deformation of vascular wall. The maximums of these parameters are revealed in first post-extrasystolic contruction in case of extrasystoles before the mitral valve opening and before the transmitral peak flow. In atrial fibrillation the main danger are the first ventricular contractions after the maximum time pauses. It causes the significant increase of cardiac output, arteries diameter as well as non-stability of atheromas and mural thrombus fragmentation with high embolism probability.



Abstract P371 Figure.

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Heparin binding copolymer reverses the anticoagulant activity of low molecular weight heparins: safety and efficacy data in rats

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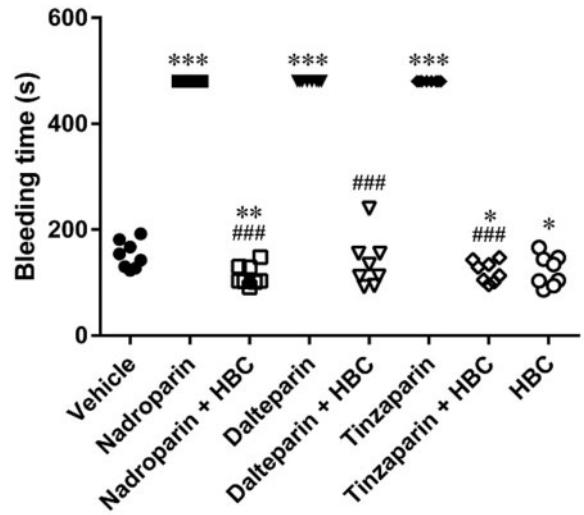
Background: Protamine, the only registered antidote of unfractionated heparin (UFH), may cause unacceptable toxicity. We developed heparin binding copolymer (HBC), a new synthetic agent directly binding UFH, enoxaparin and fondaparinux, and neutralizing their anticoagulant effect in animal models. However, it is necessary to explore a possible application of HBC to reverse the effects of other low molecular weight heparins (LMWHs), and exclude the potential toxicity before first use in humans.
Purpose: Our aim was to evaluate the safety profile of HBC and its efficacy against tinzaparin, dalteparin and nadroparin in rats.
Methods: The in vitro neutralization of tinzaparin, dalteparin and nadroparin was evaluated by measuring anti-factor Xa activity (anti-Xa). The in vivo neutralization was evaluated by measuring the time of bleeding from male Wistar rats tail (N=70). The tinzaparin (10 mg/kg), dalteparin (800 U/kg) and nadroparin (800 U/kg) were injected alone or followed by intravenous infusion of HBC (20 mg/kg). Blood samples were taken from the heart for anti-Xa activity estimation after measuring of bleeding time. HBC was incubated for 72 hours with human umbilical vein endothelial cell lines (HUVEC) to investigate potential in vitro vascular cytotoxicity. The maximum tolerated dose (MTD) of HBC studies were performed in Wistar rats (N=20), by 4-days postdose observation for clinical signs of toxicity and mortality/morbidity. HBC was administered intravenously in doses: 5, 10, 20, 40 and 80 mg/kg until MTD was determined. On the last day of MTD experiment rats were sacrificed and gross necropsy was performed. Additionally, the possible acute toxicity of HBC (6, 20, 40 mg/kg) was assessed by one-hour monitoring of blood pressure, heart rate, body temperature, oxygen saturation, perfusion and respiratory rate in male Wistar rats (N=32). All experiments involving animals were approved by Local Ethical Committees.
Results: HBC completely neutralized the anticoagulant activity of tinzaparin, dalteparin and nadroparin at in vitro conditions. Anticoagulants prolonged bleeding time, but infusion of HBC restored this parameter to baseline level, as is shown in the Figure 1 (*P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001 vs vehicle, ###P $<$ 0.001 vs appropriate LMWH). HBC did not show cytotoxic effects on HUVEC (IC50=7386 nM). The MTD was estimated to be 40 mg/kg. The therapeutic doses of HBC did not influence cardiovascular and respiratory parameters of the rats.
Conclusions: HBC successfully neutralized tinzaparin, dalteparin and nadroparin at in vitro and in vivo conditions. The safety data indicates that HBC could be a novel antidote for all parenteral anticoagulants in patients who suffer a major bleeding or require emergency surgery.



Abstract P372 Figure.

P373

Macrophage, eosinophil, and mast cell extracellular traps (METs, EETs and MCETs) participate in coronary thrombus evolution after acute myocardial infarction

KR. Pertiwi[1]; OJ. De Boer[1]; DR. Pabittei[2]; C. Mackaaij[1]; RJ. De Winter[3]; AC. Van Der Wal[1]
[1]Academic Medical Center of Amsterdam, Pathology, Amsterdam, Netherlands; [2]Academic Medical Center of Amsterdam, Cardiothoracic Surgery, Amsterdam, Netherlands; [3]Academic Medical Center of Amsterdam, Cardiology, Amsterdam, Netherlands

Funding Acknowledgements: Indonesian Endowment Fund for Education
On behalf of: Cardiovascular Pathology
Background: Extracellular traps generated by neutrophils (NETs) play important roles in the formation and propagation of the atherothrombotic mass following coronary plaque disruption, and are thus presumed to contribute to the ensuing onset of acute myocardial infarction. However, other cells such as macrophages, eosinophils and mast cells, may also capable of releasing extracellular traps, which are called as METs, EETs and MCETs, respectively. The generation of these extracellular traps also marks a distinct form of cell death namely etosis.
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Method: Thrombectomy specimens obtained from 48 AMI patients were available in paraffin sections for this study. Using HE-stains, they were classified as either 25 fresh ($<$1 day old, intact erythrocytes and granulocytes), 25 lytic (1-5 days old, lytic changes) or 19 organised ($>$5 days, fibrocellular ingrowth) thrombi. Immunohistochemistry was performed to identify neutrophils (MPO), macrophages (CD68), eosinophils (EMBP) and mast cells (tryptase). NETs, METs, EETs and MCETs were visualised in double-immunostains using the cell specific antibodies in combination with anti-citrullinated histone-3 (CitH3) antibody. Single and double- immunostained cells were counted as number/mm^2 and calculated as the average numbers/mm^2 for each thrombus category.
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Conclusion: Not only neutrophils, but also macrophages, eosinophils and mast cells are able to generate extracellular traps and undergo etosis during the evolution of coronary thrombosis. Their relative participation depends on the organisation stage of the thrombus.

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Effects of simulated hyperglycemia in vitro on insulin signaling in endothelial cells

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Emerging evidence in myocytes, hepatocytes and adipocytes indicates that hyperglycemia, a major feature of type 1 diabetes (T1DM), also plays a critical role in the development of insulin resistance and progression of type 2 DM (T2DM). Insulin regulates vascular homeostasis and endothelial function but the role of hyperglycemia in the development and progression of insulin resistance in endothelial cells remains incompletely understood.
We aimed at investigating the impact of high glucose on insulin signaling in human aortic endothalial cells (HAECs). We tested the hypothesis that high glucose per se and/or through its hyperosmolar component may lead to insulin resistance by lowering the metabolic, anti-inflammatory and anti-atherogenic insulin signaling through a down-regulation of the PI3K/AKT pathway.

MACROPHAGE, EOSINOPHIL, AND MAST CELL –EXTRACELLULAR TRAPS (METS, EETS AND MCETS) PARTICIPATE IN CORONARY THROMBUS EVOLUTION AFTER ACUTE MYOCARDIAL INFARCTION

K R Pertiwi[1,2], O J de Boer[1], D R Pabittei[3], C Mackaaij[1], R J de Winter[4], A C van der Wal[1]

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PURPOSE

- Extracellular traps (**ETs**) are "thread-like" structures, consisting of decondensed DNA with cell proteins, which mark a distinct form of cell death, namely **ETOSIS**.
- They are firstly identified as neutrophil extracellular traps (**NETs**), however other leucocytes may also generate extracellular traps, i.e. macrophages (**METs**), eosinophils (**EETs**) and mast cells (**MCETs**).
- We previously found that NETs participate in all types of coronary atherosclerotic plaque complication and associate with coronary thrombus organization.
- The potential role of METs, EETs and MCETs in human atherosclerothrombosis is not yet known.

We investigated the formation and relative extent of METs, EETs, MCETs in human coronary thrombectomy specimens in relation to the age of the thrombus.

METHODS

- Aspirated thrombus from forty-eight AMI patients were fixed in formalin, embedded in paraffin, cut into five-µm-thickness sections and graded in H&E-stains (**Figure 1**).
- Virtual multiple immunohistochemical staining was performed with anti- myeloperoxidase/MPO (neutrophils), CD68 (macrophages), eosinophil major basic proteins/EMBP (eosinophils), tryptase (mast cells) and citrullinated-histone3/ CitH3 (ETs) (**Figure 2**).
- Co-localisation of immunopositive cells were quantified using Fiji/Image J and expressed as number of cells/mm^2
- Statistical analysis was performed with SPSS 24.00.

Figure 2. Illustration of virtual multiple immunohistochemical staining procedure

Declaration of interest : nothing to declare

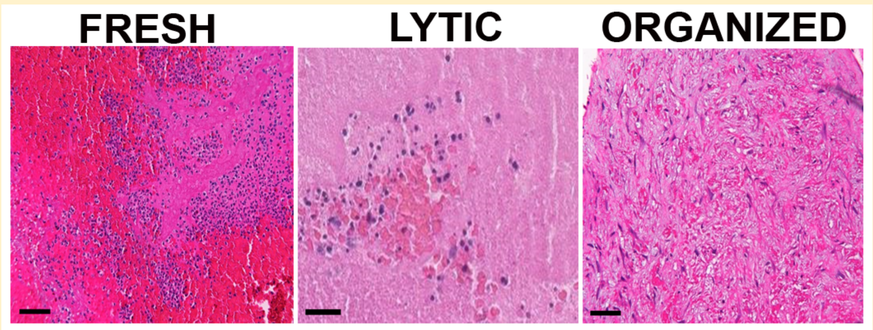

Figure 1. Classification of thrombus age (H&E-stains).
Fresh: <1 day, RBCs, platelets and granulocytes.
Lytic: 1-5 days old, necrosis and karryorhexis of granulocytes.
Organized: >5 days old, SMCs, fibromixoid matrix, collagen and capillaries.
Scale bar: 100 µm (fresh&organized) and 50 µm (lytic).

- There were 24 fresh, 26 lytic and 18 organized thrombi used in this study.
- All types of traps were observed in different thrombus ages (**Figure 3**) although their extent varied (**Figure 4**).
- The major source of traps is neutrophils, followed by macrophages; whereas EETs and MCETs were rarely observed.
- NETs were more prominent in fresh, METs and EETs in lytic and MCETs in organized thrombi.
- Interestingly, METs were more numerous than NETs in organised thrombi.

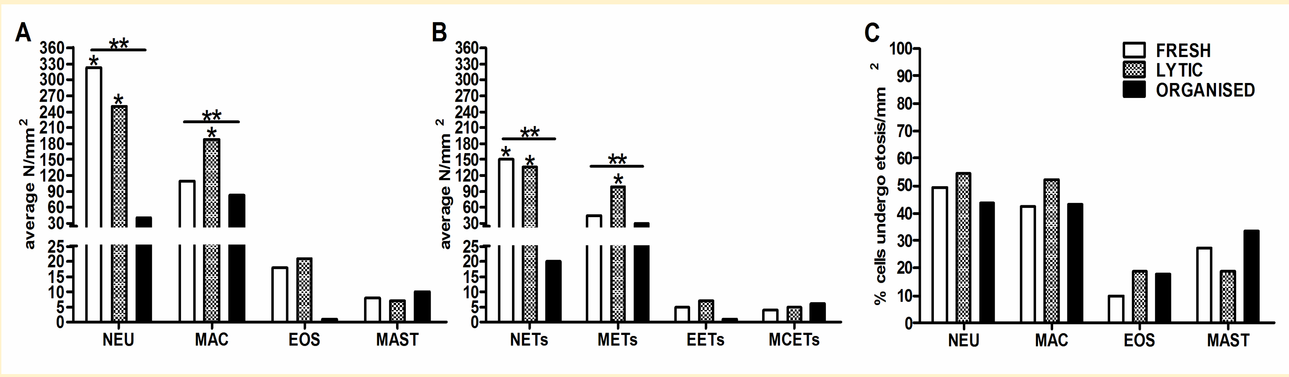

Figure 4. Quantification of NETs, METs, EETs and MCETs in different thrombus ages.
Graphs showing the number of immunopositive- cells per surface area (mm^2), stained for neutrophils/neu, macrophages/mac, eosinophils/eos and mast cells/mast (**A**); the number of (colocalised) immunopositive- cells with anti- ETs: NETs, METs, EETs and MCETs (**B**); and the percentages of cells undergo etosis (C) in relation to the age of coronary thrombi. **: p<0.05, n=68; * significant to organized thrombi.

RESULTS

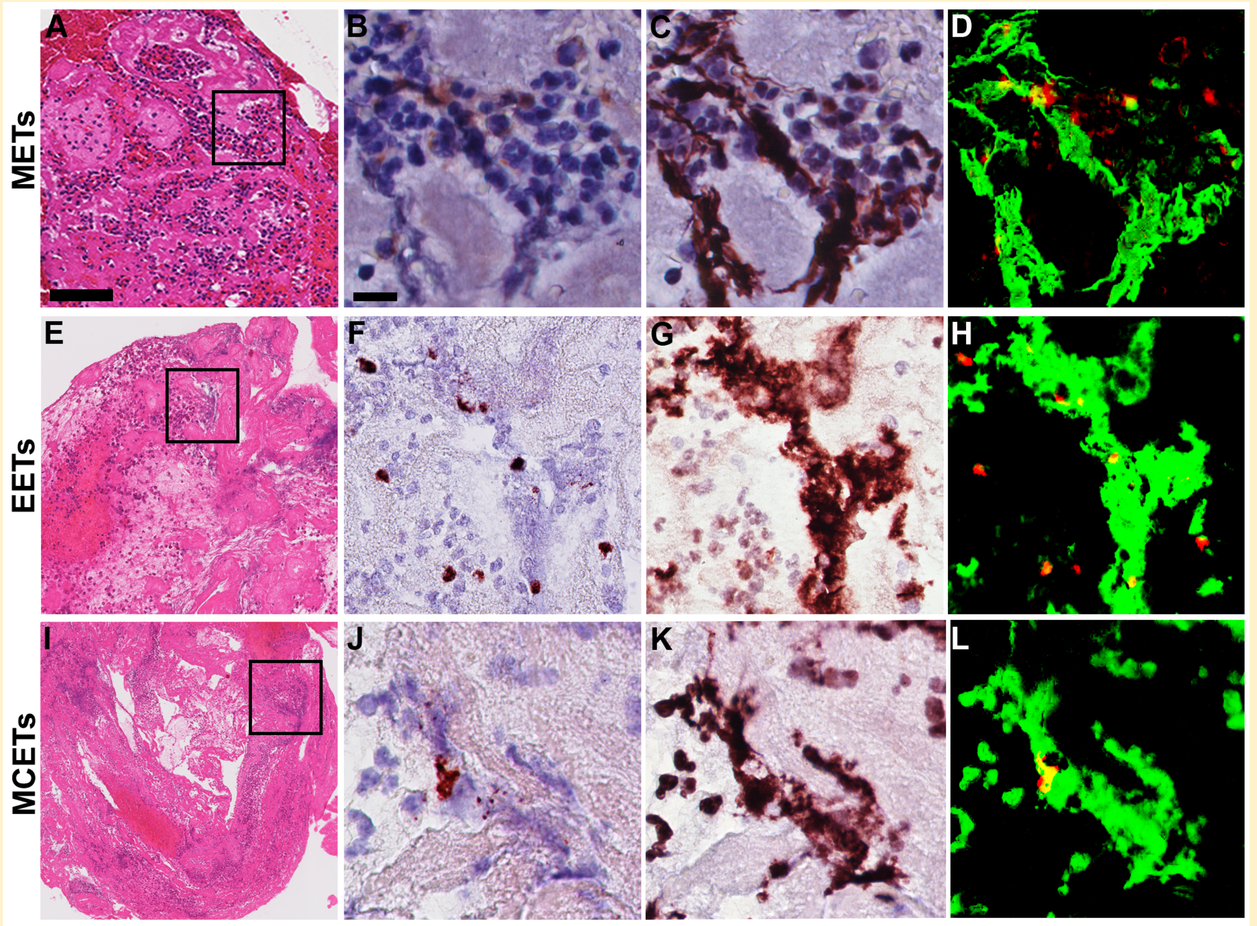

Figure 3. METs, EETs and MCETs in coronary thrombi.
Boxed areas in H&E stains (A , E and I) showing the regions of interest) for higher magnification of immunostaining and false-colour images. Immunostained cells with anti -CD68 (B), EMBP (F), tryptase (J) or CitH3 antibodies (C, G and K) are shown positive in dark red. The false-colour images show co-localisation of either CD68$^{+,}$ EMBP$^+$ or tryptase$^+$(red) with CitH3$^+$(green) in yellow, identifying METs, EETs and MCETs (D, H and L, respectively). Scale bar: 400 µm (A) and 25 µm (B).

CONCLUSIONS

- Not only neutrophils, but also macrophages, eosinophils and mast cells are the sources of ETs involved in the evolving coronary thrombosis.
- Their relative participation depends on the organisation stage of the thrombus.
- NETs and METs are the two most prominent ETs contributor in coronary atherothrombosis

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