

Randomized controlled trial protocol to investigate the antiplatelet therapy effect on extracellular vesicles in acute myocardial infarction (AFFECT EV)

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1 **Randomized controlled trial protocol to investigate the antiplatelet therapy effect on**
2 **extracellular vesicles in acute myocardial infarction (AFFECT EV)**

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1 **Abstract**

2 Activated platelets contribute to thrombosis and inflammation by the release of extracellular
3 vesicles (EVs) exposing P-selectin, phosphatidylserine (PS) and fibrinogen. P2Y12 receptor
4 antagonists are routinely administered to inhibit platelet activation in patients after acute
5 myocardial infarction (AMI), being a combined anti-thrombotic and anti-inflammatory
6 therapy. The more potent P2Y12 antagonist ticagrelor improves cardiovascular outcome in
7 patients after AMI compared to the less potent clopidogrel, suggesting that greater inhibition
8 of platelet aggregation (IPA) is associated with better prognosis. The effect of ticagrelor and
9 clopidogrel on the release of EVs from platelets and other P2Y12-exposing cells is unknown.
10 This study compares the effects of ticagrelor and clopidogrel on (i) the concentrations of EVs
11 from activated platelets (primary end-point), (ii) the concentrations of EVs exposing
12 fibrinogen, exposing PS, from leukocytes and from endothelial cells (secondary end-points)
13 and (iii) the procoagulant activity of plasma EVs (tertiary end-points) in 60 consecutive AMI
14 patients. After the percutaneous coronary intervention, patients will be randomized to
15 antiplatelet therapy with ticagrelor (study group) or clopidogrel (control group). Blood will be
16 collected from patients at randomisation, 48 hours after randomisation, and 6 months
17 following the index hospitalization. In addition, 30 age- and gender-matched healthy
18 volunteers will be enrolled in the study to investigate the physiological concentrations and
19 procoagulant activity of EVs using recently standardized protocols and EV-dedicated flow
20 cytometry. Concentrations of EVs will be determined by flow cytometry. Procoagulant
21 activity of EVs will be determined by fibrin generation test. The compliance and response to
22 antiplatelet therapy will be assessed by impedance aggregometry. We expect that plasma from
23 patients treated with ticagrelor (i) contains lower concentrations of EVs from activated
24 platelets, exposing fibrinogen, exposing PS, from leukocytes and from endothelial cells, and
25 (ii) has lower procoagulant activity, when compared to patients treated with clopidogrel.

1 AFFECT EV may identify a new mechanism of action of ticagrelor, as well as create a basis
2 for future studies to investigate whether lower EV concentrations are associated with
3 improved clinical outcomes in patients treated with P2Y12 antagonists.

4 **Keywords:** extracellular vesicles, platelets, ADP receptors, P2Y12 antagonists, ticagrelor

5

6 **Introduction**

7 Activation and aggregation of platelets on a ruptured or eroded atherosclerotic plaque
8 leads to acute myocardial infarction (AMI) [1]. During AMI, activated platelets release
9 proinflammatory mediators and bind to leukocytes, leading to leukocyte activation [1]. In
10 addition, activated platelets release fragments of their outer cell membrane, called platelet-
11 derived extracellular vesicles (EVs) [2]. A transmission electron microscope image of EVs
12 from human plasma is shown in Figure 1.

13 Platelet EVs are nanoparticles surrounded by a phospholipid membrane which
14 contains platelet-derived proteins [3]. The presence of glycoprotein IIb/IIIa (CD41/CD61)
15 enables to establish the cellular origin of EVs released from platelets or megakaryocytes
16 among other EVs present in blood [4]. If EVs expose the glycoprotein IIb/IIIa along with P-
17 selectin (CD62P) and/or phosphatidylserine (PS), or if EVs expose fibrinogen, such EVs are
18 released from activated platelets and/ or platelet-rich thrombi [5,6].

19 Platelet EVs exposing P-selectin, PS and/ or fibrinogen are likely involved in
20 inflammation and thrombosis [7-10], and potentially contribute to the development and
21 progression of atherosclerosis [10-12]. P-selectin mediates binding of platelets and platelet
22 EVs to monocytes via P-selectin glycoprotein ligand-1 on the monocyte, which leads to
23 monocyte activation, cytokine release and exposure of tissue factor (TF) [7]. PS along with
24 other negatively charged phospholipids binds clotting factors in the presence of calcium ions,
25 thereby propagating thrombin generation [8]. Fibrinogen binds both to the CD11b/CD18

1 receptor (Mac-1) on monocytes, thereby activating monocytes, and to activated glycoprotein
2 IIb/IIIa, thereby enabling platelet crosslinking and aggregation [9].

3 Results of the Canakinumab Anti-inflammatory Thrombosis Outcomes (CANTOS)
4 study showed that inhibition of the interleukin-1 β pathway decreases the rate of recurrent
5 cardiovascular events in patients after AMI, compared to placebo [13]. CANTOS proved that
6 atherosclerosis is an ongoing inflammatory disease, indicating the need for treatment
7 strategies that reduce inflammation and improve cardiovascular outcome. At present, patients
8 after AMI receive a multitude of drugs, of which the anti-inflammatory effects are not or
9 incompletely known. Drugs routinely administered after AMI include antagonists of the
10 P2Y12 receptor for adenosine diphosphate (ADP), such as ticagrelor and clopidogrel, which
11 prevent recurrent cardiovascular events in patients after AMI [14]. Ticagrelor inhibits platelet
12 activation more than clopidogrel, being a more potent antiplatelet drug [15]. Since activated
13 platelets contribute both to inflammation and thrombosis, P2Y12 receptor antagonists offer an
14 opportunity for a combined anti-inflammatory and anti-thrombotic strategy after AMI.

15 The P2Y12 receptor is exposed on platelets [16], leukocytes [17,18] and vascular
16 endothelial cells [19,20]. Binding of ticagrelor or clopidogrel to platelet P2Y12 receptors
17 increases the intracellular concentration of cyclic adenosine monophosphate, thereby making
18 platelets less sensitive to activation by other agonists [16]. If platelets are less sensitive to
19 activation, they are expected to expose and release less proinflammatory mediators, such as P-
20 selectin and CD40 ligand (CD40L), and to release less EVs. Because the more potent
21 antiplatelet drug ticagrelor improves the cardiovascular outcome of patients after AMI
22 compared to the less potent clopidogrel, greater inhibition of platelet aggregation (IPA) seems
23 associated with better prognosis [21]. However, both ticagrelor and clopidogrel decrease the
24 exposure of P-selectin, and concentrations of soluble P-selectin and CD40L to a similar
25 extent, both in patients with AMI and in healthy individuals administered endotoxin [22-25].

1 Reliable analysis of platelet P-selectin can only be performed in freshly collected whole
2 blood, i.e. within 15 minutes following blood collection [26]. Soluble P-selectin and CD40L,
3 in turn, can be released also from cells other than platelets [26,27]. Therefore, measurement of
4 P-selectin exposure on platelets and soluble platelet-derived molecules do not reflect the
5 extent of platelet activation *in vivo*.

6 Because platelet EVs are platelet-specific and can be analysed in biorepositories,
7 platelet EVs are advantageous over other available biomarkers of platelet activation [28,29].
8 Both ticagrelor and clopidogrel inhibit the release of platelet EVs [30,31], but the effects of
9 ticagrelor and clopidogrel on EVs have never been compared in a randomized clinical study.
10 We hypothesize that plasma from patients treated with ticagrelor (i) contains lower
11 concentrations of EVs from activated platelets, exposing fibrinogen, exposing PS, from
12 leukocytes and endothelial cells, and (ii) has lower procoagulant activity, when compared to
13 patients treated with clopidogrel. We aim to compare the effects of ticagrelor and clopidogrel
14 on plasma concentrations of different EV subtypes and plasma EV procoagulant activity in
15 patients with AMI.

16

17 **Methods**

18 *Study design*

19 This is a prospective, single-centre, randomised, investigator-blinded, parallel group
20 design study conducted at the 1st Chair and Department of Cardiology, Medical University of
21 Warsaw, Poland in collaboration with the Vesicle Observation Centre, and Laboratory of
22 Experimental Clinical Chemistry, Amsterdam University Medical Centres, The Netherlands.
23 The duration of this study is expected to be 12 months.

24 *Selection of participants*

1 The study inclusion and exclusion criteria are listed in Table 1. Patients will be
2 enrolled by the principal investigator among those who were admitted to 1st Chair and
3 Department of Cardiology, Medical University of Warsaw due to the first ST-segment
4 elevation acute myocardial infarction (STEMI) or non-STEMI (NSTEMI), and then
5 underwent percutaneous coronary intervention (PCI) with stent implantation. Since the
6 majority of patients with STEMI is pre-treated with clopidogrel prior to hospital admission,
7 initially all patients will be administered a loading dose of clopidogrel (600 mg) to obtain a
8 homogenous patient group. Since the reported concentrations of EVs in plasma of healthy
9 volunteers differ between studies [28], 30 age- and gender-matched healthy volunteers will be
10 enrolled in the study to investigate the physiological concentrations and procoagulant activity
11 of EVs using recently standardized protocols and EV-dedicated flow cytometry [29,32,33],
12 confirming the results of previous studies [34-36]. The principal investigator will describe all
13 study procedures and all patients and healthy volunteers will provide written informed
14 consent.

15 *Randomisation and blinding*

16 Following written informed consent, patients who meet all inclusion criteria and do
17 not meet any of the exclusion criteria will be included and randomised. Block randomisation
18 with fixed block size of size 8 without stratification will be carried out using sealed envelope
19 system in a 1:1 ratio either to replace clopidogrel with ticagrelor (study group) or to continue
20 the treatment with clopidogrel (control group). Study drugs will be administered based on the
21 randomisation list. Both randomisation and administration of the drugs will be conducted by
22 an independent operator (K.P.), not involved in sample collection and analysis. During the
23 clinical trial participants will be identified solely by an individual randomisation number. All
24 collected samples will be coded with a unique number and analysed in one block by an
25 operator blinded to patient- and treatment-related data (A.G).

1 *Treatment arms*

2 All drugs will be administered orally. Patients randomised to switch to ticagrelor will
3 receive a loading dose of ticagrelor (180 mg), followed by a maintenance dose (90 mg twice
4 daily) [14]. Patients randomised to clopidogrel will continue the treatment with a maintenance
5 dose of clopidogrel (75 mg once daily) [14]. At hospital discharge, patients (i) will receive
6 either ticagrelor or clopidogrel for 6 months of treatment and (ii) will be advised to contact
7 the principal investigator in case of recurrent thrombotic event or bleeding. Patients who
8 experience a recurrent thrombotic or bleeding event will be admitted to 1st Chair and
9 Department of Cardiology for thorough examination, assessment and monitoring. In case of
10 suspected or confirmed non-responsiveness to the initial P2Y12 antagonist, patients will
11 switch to another P2Y12 antagonist. At 6 months, all patients will be invited for the follow-up
12 visit. At the follow-up visit, compliance will be checked by counting of tablets, and ticagrelor
13 will be recommended for the remaining 6 months of double antiplatelet therapy (altogether 12
14 months of treatment, as recommended by the guidelines of European Society of Cardiology)
15 [14]. Patients who choose to switch to clopidogrel at the follow-up visit due to financial
16 constraints or other reasons will be prescribed clopidogrel.

17 *Trial schedule*

18 The trial schedule is presented in Figure 2. Venous blood will be collected from
19 fasting patients (i) 24 hours after administration of clopidogrel (randomisation), (ii) 48 hours
20 following randomisation to ticagrelor or clopidogrel group (matching the length of the
21 hospital stay of patients with AMI), and (iii) 6 months following the index hospitalization
22 (follow-up visit). Venous blood will also once be collected from 30 gender- and age-matched
23 fasting healthy volunteers.

24 Blood will be collected and processed according to the recently standardized protocol
25 for collection, handling and storage of human plasma for analysis of EVs [29]. Briefly, blood

1 will be collected using a 19-gauge needle to 10 ml plastic tube containing citrate (final
2 concentration 0.109 mol/L). The tourniquet will be removed promptly after the venepuncture.
3 The first 2.5 mL of collected blood will be discarded to avoid pre-activation of platelets. To
4 remove cells, blood will be centrifuged twice using a Rotina 380 R centrifuge equipped with
5 a swing-out rotor and a radius of 155 mm (Hettich Zentrifugen, Tuttlingen, Germany). The
6 centrifugation parameters will be 2,500 g for 15 minutes at 20°C, acceleration speed 1, no
7 brake. After the first centrifugation, plasma will be transferred to a new 5 ml plastic tube,
8 leaving ~1 cm plasma above the buffy layer. After the second centrifugation, cell- and
9 platelet-free plasma (PFP) will be collected and transferred carefully to a new 5 ml plastic
10 tube, leaving ~100 µL at the bottom of the old tube. PFP will be mixed with a pipet and 250
11 µl aliquots will be made in 1.5-ml tubes. PFP will be stored in -80°C freezer until analysed.
12 Prior to the analysis, frozen samples will be transported on dry ice to the Vesicle Observation
13 Centre, Amsterdam University Medical Centres, The Netherlands. Since EV concentrations
14 are affected by numerous pre-analytical variables, such as diameter of a needle, type of tube
15 (plastic/ glass), type of system (free flow/ vacuum), use of tourniquet (released promptly/ kept
16 during the collection), blood will be collected and processed only by operators who
17 underwent an appropriate training with subsequent quality check of the samples in LEKC,
18 Amsterdam UMC (C.E., M.B.).

19 Analyses will compare (i) the concentrations of EVs from activated platelets, exposing
20 fibrinogen, exposing PS, from leukocytes and endothelial cells, and (ii) the procoagulant
21 activity of plasma EVs between patients treated with ticagrelor and clopidogrel at 48 hours
22 and at 6 months. In addition, IPA will be assessed in all patients at randomisation, 48 hours
23 and 6 months to check the compliance and the response to ASA and P2Y12 antagonists. Data
24 regarding demographic characteristics, co-morbidities and concomitant pharmacotherapy will

1 be collected, and thorough clinical examination will be conducted during the index
2 hospitalization and at the follow-up visit.

3 *Concomitant medications*

4 If not administered prior to hospital admission, patients will receive a loading dose of
5 aspirin (300 mg) prior to PCI. Unfractionated heparin will be administered during PCI in the
6 dosage left at the discretion of the interventional cardiologist. At hospital discharge, all
7 patients will receive treatment with ASA 75 mg once daily (as part of the prescribed double
8 antiplatelet therapy) and atorvastatin at least 10 mg once daily.

9 *Analytical methods*

10 Concentration and composition of EVs

11 Concentrations of different EV subtypes, defined as a number of EVs (exceeding the
12 detection limit) per ml plasma, will be determined by EV-dedicated flow cytometry (A60
13 Micro, Apogee Flow Systems, UK) according to the recent guidelines [32,33]. EVs released
14 from different cells will be defined as following events: activated platelets: CD61⁺/P-
15 selectin⁺; fibrinogen-exposing EVs: CD61⁺/fibrinogen⁺; PS-exposing EVs: PS⁺; leukocytes:
16 CD45⁺; endothelial cells: CD31⁺/CD146⁺. Scatter detectors will be calibrated with Rosetta
17 Calibration (Exometry, The Netherlands), fluorescence detectors will be calibrated with
18 SPHERO PE Calibration kit (ECFP-F2-5K, Spherotech), Quantum FITC-5 MESF beads
19 (555A, Bangs Laboratories) and Quantum APC MESF beads (823A, Bangs), and the flow
20 rate will be calibrated with TruCount beads (BD Biosciences, Franklin Lakes, NJ, USA). To
21 enable data comparison, detection limits of scatter and fluorescence detectors will be
22 quantified in units of nm² and mean equivalent soluble fluorophore (MESF), respectively.
23 Flow-SR will be applied to determine the size and refractive index of particles and improve
24 specificity by enabling label-free differentiation between EVs and lipoprotein particles [37].

25 Procoagulant activity of EVs

1 The procoagulant activity of EVs will be determined as the ability of EVs in platelet-
2 free plasma to generate fibrin, as described previously [30]. Briefly, after pre-incubation for 5
3 min at 37°C, clotting will be initiated by addition of CaCl₂. Fibrin formation over 1 hour will
4 be determined by measuring the optical density ($\lambda = 405$ nm) in duplicate on a
5 spectrophotometer at 37°C. Because TF is the key initiator of the coagulation [38], and
6 because plasma EVs in patients with AMI expose TF [39], the procoagulant activity of plasma
7 EVs will be evaluated in the absence and presence of antibody against human TF (coagulation
8 factor VII). Recombinant human TF will be used as a positive control, and saline will be used
9 as a negative control.

10 Inhibition of platelet aggregation

11 IPA in response to double antiplatelet therapy (acetylsalicylic acid and P2Y₁₂
12 antagonist) will be assessed by impedance aggregometry (Multiplate Analyzer, Roche
13 Diagnostics) using the ASPI test (arachidonic acid, 0.5 mM) and the ADP high sensitivity test
14 (ADP, 6.5 μ M with addition of prostaglandin E₁, 9.4 nM), respectively [41].

15 *Endpoints*

16 Study end-points refer to two patient groups (ticagrelor vs clopidogrel). The primary
17 endpoint is the concentrations of EVs from activated platelets at 6 months. The secondary
18 endpoints are (i) the concentration of EVs from activated platelets at 48 hours, and (ii) the
19 concentrations of EVs exposing fibrinogen, exposing PS, from leukocytes and endothelial
20 cells at 48 hours and 6 months. The tertiary outcome is the procoagulant activity of the total
21 plasma EVs at 48 hours and 6 months. The study is not powered for mortality and other
22 adverse events.

23 *Sample size*

24 Because insufficient data are available to assess the impact of ticagrelor and
25 clopidogrel on the concentrations of platelet EVs and EVs from other types of cells in patients

1 with AMI, the standard deviation (SD) and mean difference between the two treatment arms
2 was estimated based on the preliminary *in vitro* experiments, which we conducted during
3 preparation for this study [30]. In our experiments, ticagrelor decreased ADP-induced platelet
4 EVs release 3-fold, with the SD ± 1.0 value of the mean [30]. It was not feasible to investigate
5 the effect of clopidogrel on EV release *in vitro* due to the instability of the clopidogrel active
6 metabolite. However, data from the literature show that clopidogrel decreases the release of
7 platelet EVs *ex vivo* by 2-fold, with SD ± 1.0 [31]. The required sample size was calculated
8 by a two-sided t test at a significance level of 0.05 with the following assumptions: (i) SD in
9 each group ± 1.0 , (ii) mean difference between the groups = 1, (iii) nominal test power = 0.9.
10 Based on this sample size estimation, a total of 46 patients (23 per group) should be enrolled
11 in the trial. Since 5 to 30% of patients in clopidogrel group may have inadequate platelet
12 inhibition [41], requiring to switch to a more potent P2Y12 receptor antagonists in case of
13 recurrent thrombotic event, and since the overall rate of switching between P2Y12 antagonists
14 reported in registries ranges from 5% to 50% [42,32], we assumed that up to 30% of patients
15 may be potentially lost to follow-up. Based on this assumption, we estimated that 60 patients
16 (30 per group) should be enrolled in the trial.

17 *Statistical plan*

18 A single statistical analysis will be performed at the end of the study using IBM SPSS
19 Statistics 20. Since the differences between healthy volunteers and controls will be reported
20 descriptively, no statistical matching of healthy volunteers and patients will be performed.
21 Intention to treat (ITT) and per protocol populations will be defined. The ITT population will
22 consist of all patients who were randomized and received at least one dose of the study drug,
23 regardless of protocol violations. The per protocol population will consist of all patients who
24 were randomized and treated completely in accordance with the study protocol. Categorical
25 variables will be presented as number and percent; continuous variables will be presented as

1 mean and SD or median with inter-quartile range. Data will be displayed graphically for
2 visual examination. Shapiro-Wilk test will be used to test for non-Gaussian distribution of
3 continuous variables. No formal statistical testing will be performed on the differences
4 between the two treatment arms among patients with AMI at randomisation. Linear regression
5 with EV concentration at randomisation as an addition co-variate will be used to compare the
6 concentrations between the two treatment arms at 48 hours and 6 months. Student's two-sided
7 t-test or Mann–Whitney U test will be used to compare the EV procoagulant activity between
8 the two treatment arms at 48 hours and 6 months. No corrections for multiple testing will be
9 performed. Pearson or Spearman correlation coefficient will be used to analyse a correlation
10 between IPA and EV concentrations in both study groups, separately in patients responding
11 and not-responding to clopidogrel, according to the impedance aggregometry results. The
12 non-responsiveness to clopidogrel will be defined as platelet aggregation > 46 aggregation
13 units in response to 6.5 μ M ADP [44]. A p-value below 0.05 will be considered significant.
14 Mortality and other adverse events as well as comparison of EV concentrations and EV
15 procoagulant activity between healthy volunteers and all AMI patients will be reported
16 descriptively.

17 *Legal considerations*

18 The study protocol was approved by the Bioethical Committee Approval of Medical
19 University of Warsaw (approval number: KB/112/2016), and registered in the ClinicalTrials
20 database (NCT02931045). Recording of adverse events will be conducted according to good
21 clinical practice, the ethical principles described in the Declaration of Helsinki, the
22 requirements of the European Medicines Agency, and local legal and regulatory requirements.
23 Data storage will be performed in accordance with local data protection laws. Competent
24 authorities and sponsor authorised persons may request access to trial documentation in case
25 of an inspection or audit. Direct access to these documents must be guaranteed by the

1 principal investigator. Documentation can be copied during inspection or audit in case the
2 identity of the participant have been made unrecognisable.

3

4 **Expected results**

5 We expect lower concentrations of EVs from activated platelets, exposing fibrinogen,
6 exposing PS, from leukocytes and endothelial cells, as well as lower procoagulant activity of
7 plasma EVs in patients treated with ticagrelor, compared to clopidogrel. Consequently, we
8 expect to identify a new mechanism of action of ticagrelor. If present, this mechanism would
9 create a basis for future studies to investigate whether lower EV concentrations are associated
10 with improved clinical outcomes in patients treated with P2Y12 antagonists.

11 We expect higher concentrations of all EV subtypes in patients with AMI at
12 randomisation, compared to healthy volunteers, thereby confirming using the results of
13 preceding studies [31-33] using recently standardized protocols and EV-dedicated flow
14 cytometry [28-30].

15

16 **Discussion**

17 AFFECT EV is the first study which directly compares the effects of ticagrelor and
18 clopidogrel on the concentrations and procoagulant activity of EVs in patients with AMI in a
19 randomised and investigator-blinded way. The state-of-the art methods to collect and handle
20 samples and to analyse PEVs will account for the reliability of results. The translational
21 collaboration between the 1st Chair and Department of Cardiology, Medical University of
22 Warsaw, Poland, and the Vesicle Observation Centre, Amsterdam University Medical
23 Centres, The Netherlands will further ensure scientifically sound interpretation of data and
24 optimize dissemination of results.

1 AFFECT EV is expected to establish whether EVs can be *affected* by drugs routinely
2 used in patients after AMI, thereby identifying one of the potential novel mechanisms of
3 action contributing to a combined anti-thrombotic/ anti-inflammatory benefit of the P2Y12
4 receptor antagonists. Regarding the exponentially growing interest in EVs during the last
5 decade [30], determining the effect of the P2Y12 receptor antagonists on EVs may be the first
6 step to explain the clinical benefits of long-term treatment with P2Y12 antagonists, and may
7 provide a basis for future studies aimed to associate this effect with clinical outcomes.

8

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14

15 **Declaration of Interest**

16 E. van der Pol is a cofounder and shareholder of Exometry BV. All other authors report no
17 declarations of interest.

18

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24

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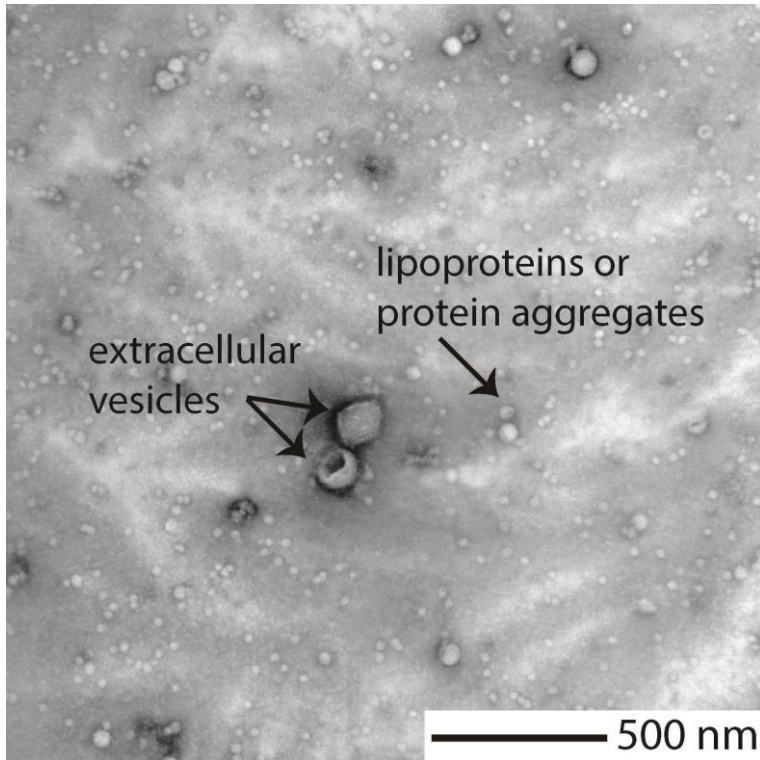
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1 **Tables and figures**

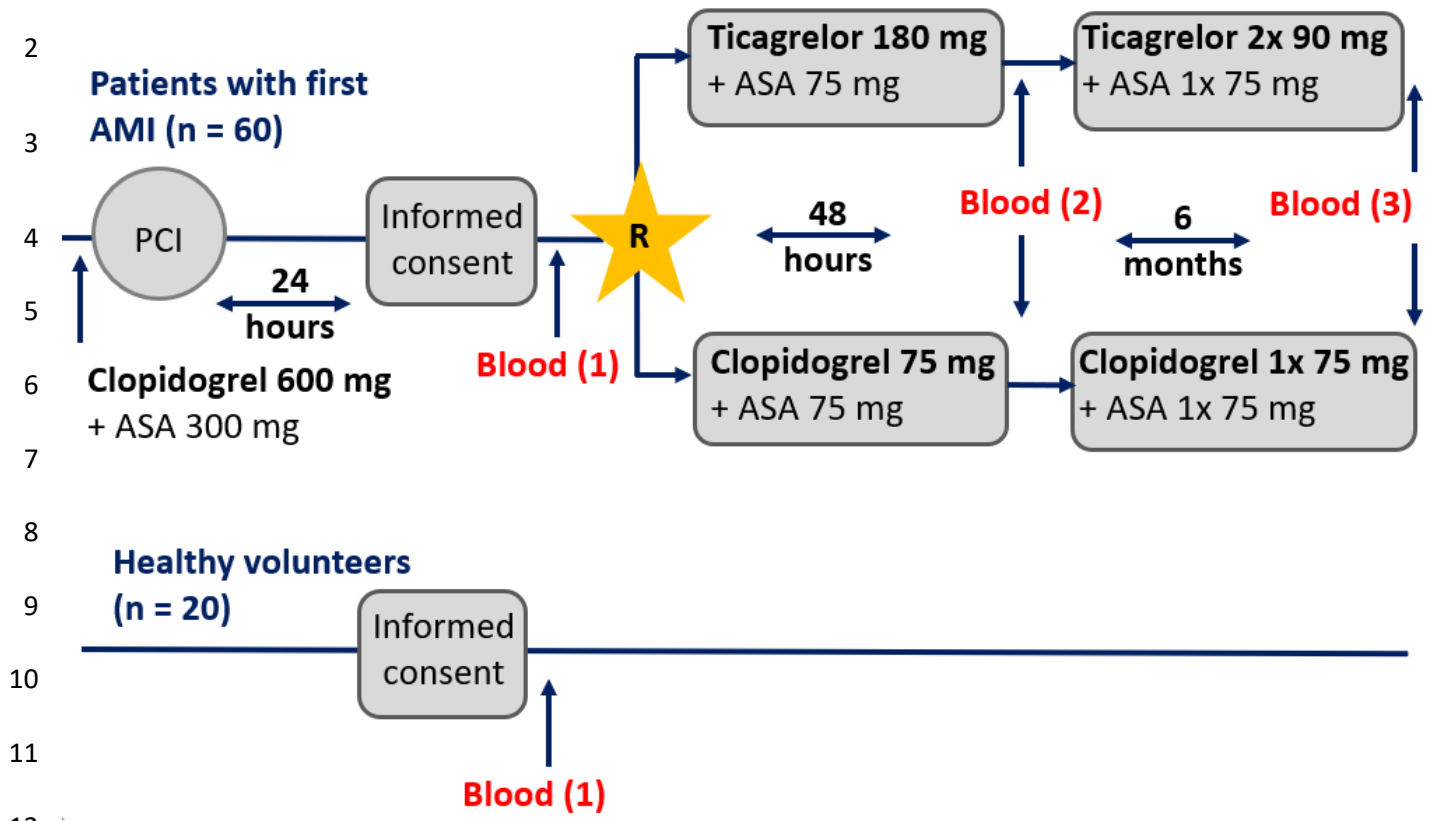
2 Figure 1. Transmission electron microscopy image of extracellular vesicles in human plasma.

3 Image courtesy Linda G. Rikkert, Vesicle Observation Centre, Academic Medical Centre of

4 the University of Amsterdam.



1 Figure 2. Trial schedule.



13 Abbreviations: AMI – acute myocardial infarction; ASA – acetylsalicylic acid; PCI –
14 percutaneous coronary intervention; R – randomization.

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1 Table 1. Eligibility criteria for the study

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> • Age > 18 years • Informed consent to participate in the study • First ST-elevation myocardial infarction (STEMI) or non-STEMI (NSTEMI) • Percutaneous coronary intervention (PCI) with stent implantation • Administration of the loading dose of clopidogrel (600 mg) prior to PCI 	<ul style="list-style-type: none"> • Known coagulopathy • Active pathological bleeding • Known history of bleeding disorder • Suspicion of intracranial haemorrhage • Need for oral anticoagulation therapy • Administration of GPIIb-IIIa antagonists • Cardiogenic shock • Severe chronic renal failure (eGFR < 30 mL/min) • Severe liver insufficiency • Infectious disease • Autoimmune disease • Neoplasm • Chronic dyspnea • Increased risk of bradycardia • Known pregnancy, breast-feeding, or intention to become pregnant during the study period • Study drug intolerance • Co-administration of ticagrelor or clopidogrel with strong CYP3A4 inhibitors • Participation in any previous study with ticagrelor or clopidogrel

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