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# Platelet reactivity during mild therapeutic hypothermia in patients with acute myocardial infarction treated with ticagrelor: study protocol of a single-centre study

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## ABSTRACT

In summary, the available data on the antiplatelet efficacy of P2Y<sub>12</sub> receptor inhibitors suggest their less potent and/or delayed effect in patients undergoing mild therapeutic hypothermia (MTH). However, previous studies do not explain the mechanisms of the impact of MTH on platelet function. Moreover, there is a lack of evidence of any relationship between the increased prevalence of thrombotic complications in MTH patients and the anti-platelet effect of P2Y<sub>12</sub> receptor inhibitors.

We hypothesise that MTH may interfere with the absorption of ticagrelor and consequently results in significant changes in the pharmacokinetic and pharmacodynamic profile of this P2Y<sub>12</sub> receptor inhibitor. It cannot be ruled out that the high initial level of platelet activation in MTH patients may additionally modify the pharmacodynamics of ticagrelor. Malabsorption may slow down the desired antiplatelet effect, while high levels of platelet activation may reduce the antiplatelet effect of ticagrelor.

The aim of this study is to verify whether the use of MTH after resuscitation in out-of-hospital cardiac arrest (OHCA) patients with STEMI (ST-segment elevation myocardial infarction) treated with primary percutaneous coronary intervention (pPCI), affects the anti-platelet effect of ticagrelor. Moreover, we attempt to elucidate the mechanisms of impaired effect of ticagrelor in MTH patients based on pharmacodynamic and pharmacokinetic measurements.

To achieve the aim of the study, we planned the following: 1) comparison of the pharmacokinetic and pharmacodynamic results obtained from the study population (MTH + pPCI + ticagrelor) with results obtained from a demographically and clinically comparable population of patients with STEMI treated with primary PCI and receiving ticagrelor (pPCI + ticagrelor); and 2) analysis of the pharmacodynamic results in relation to the pharmacokinetic measurements in the target population.

Understanding the mechanisms standing behind the impact of MTH on the efficacy of platelet inhibition with P2Y<sub>12</sub> inhibitors is pivotal in reducing the risk of thrombotic complications. The study is expected to provide information leading to improvement of the safety of MTH in STEMI patients with OHCA treated with pPCI and receiving ticagrelor.

**Key words:** platelet reactivity, ticagrelor, hypothermia

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## Introduction

Platelet activation plays a key role in the pathophysiology of acute coronary syndromes, including myocardial infarction with ST-segment elevation (STEMI). Pharmacological platelet inhibition with P2Y<sub>12</sub> receptor antagonists and acetylsalicylic acid is essential for the treatment and prevention of thrombotic complications in patients with STEMI undergoing primary percutaneous coronary intervention (pPCI) [1–3].

The results of the PLATO study showed that treatment with ticagrelor, a new potent inhibitor of the platelet P2Y<sub>12</sub> receptor, reduces the overall mortality and cardiovascular thrombotic events, as compared with clopidogrel, in patients with acute coronary syndromes (ACS), including STEMI [1]. The superiority of ticagrelor over clopidogrel in patients with ACS results from faster, more potent, and more uniform action of the former versus the latter of these drugs [1]. Ticagrelor currently holds a class I recommendation, level of evidence B in patients with STEMI, both in the guidelines from the European Society of Cardiology (ESC) and from the American College of Cardiology/American Heart Association (ACCF/AHA) [2, 3].

Out-of-hospital cardiac arrest (OHCA) is the most frequent cause of sudden death in developed countries. OHCA survivors remaining in a coma despite restored cardiovascular function are saddled with a very high risk of death and serious neurological complications. The ESC and ACCF/AHA guidelines recommend the use of mild therapeutic hypothermia (MTH) in OHCA patients with myocardial infarction [2, 3]. According to the ESC guidelines [2], MTH is indicated early after resuscitation of cardiac arrest patients who are comatose or in deep sedation (Class of recommendation I, level of evidence B). Immediate angiography with a view to pPCI is recommended in patients with resuscitated cardiac arrest, whose ECG shows STEMI (class of recommendation I, level of evidence B), and it should be also considered in survivors of cardiac arrest without diagnostic ECG ST-segment elevation, but with a high suspicion of ongoing infarction. (Class of recommendation IIa, level of evidence B) [2]. According to the ACCF/AHA guidelines [3], MTH should be started as soon as possible in comatose patients with STEMI and out-of-hospital cardiac arrest caused by ventricular fibrillation (VF) or pulseless ventricular tachycardia (VT), including patients who undergo pPCI (class of recommendation I, level of evidence B). Immediate angiography and pPCI when indicated should be performed in resuscitated out-of-hospital cardiac arrest patients whose initial ECG shows STEMI (class of recommendation I, level of evidence B) [3].

Several studies, including our own [4, 5] as well as studies published by Casella et al. [6] and Dumas et

al. [7], suggest that the combination of PCI and MTH is more effective than any of these methods used alone in the treatment of OHCA patients with ACS. However, there is a serious limitation of MTH in patients with acute myocardial infarction treated with PCI as described by Penela et al. [8]. They observed clinical resistance to clopidogrel with an extremely high incidence of acute stent thrombosis. In a small group of 11 MTH patients enrolled into the study, stent thrombosis occurred in five patients, while two other patients experienced other thrombotic complications [8]. This observation was not supported by any pharmacokinetic or pharmacodynamic data that could explain the mechanisms of thrombotic complications. In another study, Ibrahim et al. [9] compared the antiplatelet effect of clopidogrel, prasugrel, and ticagrelor 24 hours after loading dose in patients treated with and without hypothermia. The authors found a significantly higher incidence of unresponsiveness to the drug (PRI/VASP > 50%) only among those patients undergoing MTH who received clopidogrel (82% vs. 26%). However, the absolute values of PRI/VASP were also significantly higher in patients undergoing MTH and treated with ticagrelor. This study [9], suggesting less potent antiplatelet effect of all oral P2Y<sub>12</sub> receptor inhibitors in patients undergoing MTH solely based on sparse pharmacodynamic data (one measurement after 24 hours), provides no information on pharmacokinetics, which would explain the mechanism of differences in platelet inhibition [9].

In summary, the available data on the antiplatelet efficacy of P2Y<sub>12</sub> receptor inhibitors suggest their less potent and/or delayed effect in patients undergoing MTH. However, previous studies [8, 9] do not explain the mechanisms of impact of MTH on platelet function. Moreover, there is a lack of evidence of any relationship between the increased prevalence of thrombotic complications in MTH patients and the anti-platelet effect of P2Y<sub>12</sub> receptor inhibitors.

We hypothesise that MTH may interfere with the absorption of ticagrelor and consequently result in significant changes in the pharmacokinetic and pharmacodynamic profile of this P2Y<sub>12</sub> receptor inhibitor. It cannot be ruled out that the high initial level of platelet activation in MTH patients may additionally modify the pharmacodynamics of ticagrelor. Malabsorption may slow down the desired antiplatelet effect, while high levels of platelet activation may reduce the antiplatelet effect of ticagrelor.

The aim of this study is to verify whether the use of MTH after resuscitation in OHCA patients with STEMI treated with pPCI affects the anti-platelet effect of ticagrelor. Moreover, we attempt to elucidate the mechanisms of the impaired effect of ticagrelor in MTH patients based on the pharmacodynamic and pharmacokinetic measurements.

To achieve the aim of the study, we have planned the following:

- comparison of the pharmacokinetic and pharmacodynamic results obtained from the study population (MTH + pPCI + ticagrelor) with results obtained from a demographically and clinically comparable population of patients with STEMI treated with primary PCI and receiving ticagrelor (pPCI + ticagrelor);
- analysis of the pharmacodynamic results in relation to the pharmacokinetic measurements in the target population.

## Methods

This PK-PD, phase IV, single-centre, investigator-initiated, prospective, observational study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol of the study was approved by the Ethics Committee of The Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz (approval number KB 339/2015). Each patient with STEMI treated with pPCI without MTH will provide written, informed consent to participate in the study before enrolment. In this group of patients, additional blood sampling is required in comparison to regular STEMI patients who do not participate in the study. In patients treated with additional MTH due to OHCA it will not be possible to obtain informed consent before blood sampling; however, these patients will require no additional sampling outside the protocol of MTH monitoring. All patients before pPCI receive a loading dose of aspirin (300 mg) and a loading dose of ticagrelor (180 mg), according to the ESC guidelines.

### Study population

Study group: 25 STEMI patients after OHCA aborted with successful resuscitation, treated with MTH, pPCI, and ticagrelor.

Control group: 25 STEMI patients treated with pPCI and ticagrelor (both populations matched regarding the demographic and clinical variables).

Due to the lack of previous relevant studies, the number of patients that allows estimation of the final sample size sufficient to detect statistically significant differences in the primary endpoint was assumed arbitrarily.

Based on a historical analysis of the number of OHCA patients with concomitant STEMI treated with pPCI, who underwent MTH therapy, the time required to enrol the assumed number of patients is estimated to be 20 months.

Inclusion criteria are shown in Table 1, and exclusion criteria are shown in Table 2.

### Induction of hypothermia

The induction of hypothermia will start upon hospital admission, directly after the initial assessment of consciousness [5], and will employ the following:

- ice packs — placed on the largest possible surface area of the patient's body: neck, armpits, abdomen, groin, lower limbs;
- infusion of cold fluid — 1–1.5 L of 4°C lactated Ringer's solution or cold normal saline infused into the peripheral veins;
- washing the patient's skin with alcohol after completion of the diagnostic and invasive procedures, and after final qualification for MTH.

### Mild therapeutic hypothermia

Mild therapeutic hypothermia will be performed and monitored in a Cardiac Intensive Care Unit. The state of MTH is defined as body core temperature below 34°C, with a target temperature of 33°C. To reach the target temperature and maintain it over the subsequent 24 hours, methods of external and intravascular cooling

**Table 1.** Inclusion criteria

INCLUSION CRITERIA	
Study group	Control group
Age > 18 years	Age > 18 years
Ecg findings suggestive of STEMI	ECG findings suggestive of stemi
Primary PCI with stenting of at least one coronary artery	Primary pci with stenting of at least one coronary artery
Survival from OHCA	
Sustained return of spontaneous circulation (ROSC) at least 20 minutes after the onset of resuscitation	
Unconsciousness (glasgow coma scale score < 8 and > 3) after ROSC (in case of prior sedative drug administration assessed after reversal of their actions with naloxone/anexate)	
Shockable initial rhythm	

ECG — electrocardiography; STEMI — ST-segment elevation myocardial infarction; PCI — percutaneous coronary intervention; OHCA — out-of-hospital cardiac arrest

**Table 2.** Exclusion criteria

EXCLUSION CRITERIA	
Study group	Control group
Overt or suspected pregnancy	Overt or suspected pregnancy
Known serious infection/sepsis before OHCA	Known serious infection/sepsis before OHCA
Known bleeding diathesis	Known bleeding diathesis
Confirmed or suspected internal bleeding	Confirmed or suspected internal bleeding
Confirmed or suspected acute stroke	Confirmed or suspected acute stroke
Confirmed or suspected cerebral injury	Confirmed or suspected cerebral injury
Known serious neurological dysfunction (CPC $\leq$ 4) before OHCA	Known serious neurological dysfunction (CPC $\leq$ 4) before OHCA
Known serious disease with survival prognosis $\leq$ 180 days	Known serious disease with survival prognosis $\leq$ 180 days
Haemodynamic instability with systolic blood pressure $<$ 65 mm Hg despite treatment	Haemodynamic instability with systolic blood pressure $<$ 65 mm Hg despite treatment
Contraindications to ticagrelor	Contraindications to ticagrelor
Administration of glycoprotein IIb/IIIa receptor inhibitor	Administration of glycoprotein IIb/IIIa receptor inhibitor
Resuscitation duration $<$ 5 min	
Time delay from ROSC to MTH induction $>$ 240 minutes	

OHCA — out-of-hospital cardiac arrest; CPC — cerebral performance categories; MTH — mild therapeutic hypothermia

will be used. Immediately after PCI an MTH-dedicated catheter will be placed in the inferior vena cava through the femoral vein under fluoroscopic guidance in the cath lab.

Besides quick arrival at the target temperature and its successful maintenance, properly conducted hypothermia is also characterised by: bradycardia of 40–50 bpm, urine output of  $>$  1 mL/kg body weight/hour, and lack of shivers.

The treatment is focused on achieving the designated haemodynamic and biochemical goals. According to the 2008 International Liaison Committee Recommendations, they include [10]:

- body core temperature in the range of  $33.0 \pm 0.2^\circ\text{C}$ ;
- mean arterial blood pressure of 65–95 mm Hg;
- central venous pressure of 8–12 mm H<sub>2</sub>O;
- blood saturation of 92–96% (90–94% for COPD patients);
- diuresis  $>$  1 mL/kg body weight/h;
- temperature-corrected normoxaemia in arterial blood sample;
- temperature-corrected normocapnia in arterial blood sample;
- potassium, calcium, and phosphorus concentrations within normal ranges;
- lactate concentration  $<$  2 mmol/L;
- blood glucose concentration of 110–170 mg/dL;
- maintenance of 24-hour fluid balance (with controlled administration of colloid/crystalloid fluids) at approx.  $-300$  to 0 mL;
- central venous oxygen saturation  $>$  70%.

### Pharmacodynamics of ticagrelor

Blood samples will be drawn at predefined time points: before administration of a 180-mg loading dose

of ticagrelor, then after: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours.

For assessment of ticagrelor pharmacodynamics four different methods will be used:

- light transmission aggregometry (LTA — 490-4D Chronolog) — ADP-induced aggregation;
- impedance aggregometry using semi-automatic analyser Multiplate — ADP-induced aggregation;
- automatic VerifyNow — P2Y<sub>12</sub> assay — ADP-induced aggregation;
- platelet vasodilator-stimulated phosphoprotein (VASP) assay using flow cytometer FASCalibur (the most specific method for the quantification of the pharmacodynamic effects of platelet P2Y<sub>12</sub> receptor inhibitors), analysed parameter: platelet reactivity index (PRI/VASP).

### Pharmacokinetics of ticagrelor and its active metabolite (AR-C124910XX)

Blood plasma concentrations of ticagrelor and AR-C124910XX will be evaluated at the same time points as pharmacodynamics, using liquid chromatography mass spectrometry.

### Basic study information

- Single-centre, prospective, observational study;
- comparing two groups of patients:
  - OHCA patients after resuscitation undergoing MTH therapy, treated with primary PCI and ticagrelor due to STEMI,
  - patients with STEMI treated with pPCI and ticagrelor;

- duration of observation: 24 hours;
- primary endpoint: ADP-induced platelet aggregation assessed with impedance aggregometry Multiplate analyser at: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours after administration of a 180-mg loading dose of ticagrelor;
- secondary endpoints:
  - ADP-induced platelet aggregation assessed with light transmission aggregometry (LTA) at: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours after administration of a 180-mg loading dose of ticagrelor.
  - platelet reactivity index (PRI/VASP) assessed with flow cytometer at: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours after administration of a 180-mg loading dose of ticagrelor,
  - ADP induced platelet aggregation assessed with VerifyNow analyzer at: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours after administration of a 180-mg loading dose of ticagrelor,
  - area under the curve of ticagrelor concentration and its metabolite AR-C124910XX levels during follow-up (AUCO- $\tau$ ),
  - maximum concentration of ticagrelor ( $C_{max}$ ) and its metabolite AR-C124910XX,
  - time to reach  $C_{max}$  of ticagrelor and its metabolite AR-C124910XX,
  - ticagrelor serum concentration at: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours after administration of a 180-mg loading dose of ticagrelor,
  - AR-C124910XX serum concentration at: 30 minutes, and 1, 2, 3, 4, 6, 12, and 24 hours after administration of a 180-mg loading dose of ticagrelor;
- clinical endpoint: stent thrombosis within 24 hours of study;
- safety endpoint: bleeding by the BARC criteria, bradyarrhythmias.

### Characteristics of the final result

Understanding the mechanisms behind the impact of MTH on the efficacy of platelet inhibition with

P2Y12 inhibitors is pivotal to reduce the risk of thrombotic complications. The study is expected to provide information allowing us to improve the safety of MTH in STEMI patients with OHCA, treated with pPCI, and receiving ticagrelor.

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