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Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): a protocol for prospective, observational, single centre study

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Keywords: NSTEMI, pharmacodynamics, pharmacokinetics, STEMI, ticagrelor

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ABSTRACT

Introduction

Ischaemic heart disease is the most common cause of death in the world and acute myocardial infarction (AMI) remains its common lethal presentation. The most often applied classification of AMI is based on electrocardiographic findings and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). Epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI. Ticagrelor is a P2Y12 receptor inhibitor which is the first line treatment in both STEMI and NSTEMI patients. Available data suggest that STEMI diagnosis can be associated with lower plasma concentrations of ticagrelor in the first hours of AMI, but currently there are no studies directly comparing ticagrelor's pharmacokinetics or antiplatelet effect in STEMI and NSTEMI patients.

Methods and analysis

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational study designed to compare pharmacokinetics and pharmacodynamics of ticagrelor in STEMI and NSTEMI patients designated to invasive strategy. Based on the internal pilot study, the trial is expected to include at least 23 patients with each AMI type. All subjects will receive 180 mg loading dose of ticagrelor. The primary end-point of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor for the first 6 hours after the loading dose of ticagrelor. Secondary end-points include various pharmacokinetic features of ticagrelor and its active metabolite (AR-C124910XX), and evaluation of platelet reactivity by VASP assay and multiple electrode aggregometry within 12 hours from ticagrelor loading dose.

Ethics and dissemination

The study received approval from the Local Ethics Committee to conduct the study (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; approval reference number KB 617/2015). The study results will be disseminated through conference presentations and publication in peer-reviewed journal.

Trial Registration: ClinicalTrials.gov identifier: NCT02602444 (November 09, 2015)

INTRODUCTION

According to the World Health Organization ischaemic heart disease is the most common cause of death in the world. In year 2012 alone it was responsible for 7.4 million deaths worldwide.[1] Current data show that ischaemic heart disease itself can be accounted for even up to 20% of all deaths in the developed or developing countries.[2]

Although the efficacy of acute myocardial infarction (AMI) treatment has substantially improved in the last decades, AMI still remains dangerous and lethal presentation of ischaemic heart disease. Third Universal Definition of Myocardial Infarction recognizes five different types of myocardial infarction based on their pathomechanism or clinical cause.[3] However, a different classification is routinely applied in everyday practice to facilitate the immediate choice of treatment strategy in AMI patients. This classification is based on the electrocardiographic findings and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI).[3]

Over the past years the incidence of STEMI has decreased, while the occurrence of NSTEMI has slightly increased, and currently STEMI and NSTEMI occur almost equally often.[4, 5] Short-term mortality is higher in STEMI patients, however the mortality rates become comparable or even higher in NSTEMI patients at long-term follow-up.[6-9]

In STEMI, which is usually caused by acute total occlusion of coronary artery, immediate primary percutaneous coronary intervention (PCI) is the mainstay of treatment.[10] In NSTEMI the therapeutic strategy and its timing depends on the risk stratification. The urgency of recommended invasive coronary evaluation in this type of AMI varies from <2 hours in very high risk patients (immediate invasive strategy), through <24 hours in high risk patients (early invasive strategy), to <72 hours in intermediate risk patients (invasive strategy).[9]

Complementary to coronary revascularization, dual antiplatelet therapy consisting of aspirin on top of a P2Y12 receptor inhibitor remains the cornerstone of pharmacological treatment in AMI patients, including both STEMI and NSTEMI. The European Society of Cardiology (ESC) guidelines recommend use of ticagrelor or prasugrel as the first line P2Y12 receptor inhibitor in patients with STEMI and NSTEMI (class of recommendation I, level of evidence B).[9, 10] Unlike prasugrel, ticagrelor can be used also in conservatively treated patients and in those who are likely to undergo coronary artery bypass grafting.

The importance of platelet P2Y12 receptor blockade in patients with AMI derives from the essential role exerted by platelet activation and aggregation in the pathophysiology of acute coronary syndromes (ACS).[11] Inadequate platelet inhibition during treatment with P2Y12 receptor inhibitors, defined as high platelet reactivity (HPR), is an important risk factor for stent thrombosis and can be associated with increased mortality.[12] Therefore, effective and rapid suppression of platelet activation is pivotal in patients with AMI treated with PCI. Noteworthy, it has been reported that platelet inhibition by ticagrelor and its active metabolite (AR-C124910XX) is proportional to their plasma concentrations.[13]

Impact of numerous clinical features on plasma concentration and pharmacodynamics of ticagrelor has been inspected. Genetic effects, gender, age, concomitant food intake or preloading with clopidogrel have at most minimal influence on pharmacokinetics of ticagrelor, which does not translate into any clinically significant differences in the degree of platelet inhibition.[14-17] On the other hand, morphine administration has been shown to affect pharmacokinetic profile as well as antiplatelet effect of ticagrelor not only in healthy volunteers, but also in AMI patients.[18-20] Negative impact of morphine on the intestinal absorption has been proposed as an explanation for the observed interactions, while no evidence was found that morphine affects ticagrelor conversion to its active metabolite.[19, 21] Moreover, multiple regression analysis of data obtained in the randomized IMPRESSION study (Influence of Morphine on Pharmacokinetics and Pharmacodynamics of Ticagrelor in Patients with Acute Myocardial Infarction) suggests that clinical presentation as STEMI when compared with NSTEMI is independently associated with lower plasma concentrations of ticagrelor.[19]

Even though ticagrelor shows more potent and prompt platelet inhibition than clopidogrel, it still fails to provide desired antiplatelet effect in the first hours after the loading dose (LD) in all STEMI patients.[22] The data on proportion of NSTEMI patients loaded with ticagrelor who are at risk of HPR during peri-PCI period is sparse, however as expected ticagrelor has been shown to provide stronger platelet blockade than clopidogrel in this clinical setting.[23] Solely a pharmacodynamic study by Laine et al. reported that platelet reactivity assessed with the platelet vasodilator-stimulated phosphoprotein (VASP) assay after administration of a 180 mg ticagrelor LD was not uniform among ACS patients, but when grouped by ACS type (STEMI, NSTEMI and unstable angina) it appeared to be similar (p=0.9). However, the authors assessed the antiplatelet effect of ticagrelor only once in each patient and the time of blood sampling differed substantially among trial participants. Additionally, blood samples for pharmacodynamic evaluation were obtained between 6 and 24 hours after ticagrelor LD, so the first crucial hours after PCI were not covered by the analysis.[24]

The PLATO (Platelet Inhibition and Patient Outcomes) trial has shown superior efficacy of ticagrelor to clopidogrel in ACS patients. This superiority was demonstrated in most of the analysed subgroups, including patients with STEMI and NSTEMI.[25] Nevertheless, epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI, while recommended dosing regimens of ticagrelor are identical in both clinical settings.[9, 10] Currently, there are no data on direct comparison of ticagrelor's pharmacokinetics in the mentioned types of AMI, while STEMI patients may be at risk of having lower plasma concentrations of ticagrelor in the most crucial time during early hours of AMI treatment.[19] Similarly, potential differences in ticagrelor's antiplatelet effects between STEMI and NSTEMI have not been defined yet. Therefore, we decided to verify whether pharmacokinetics and pharmacodynamics of ticagrelor differ between STEMI and NSTEMI patients, which we believe could provide a valuable insight into our knowledge regarding modern treatment of AMI patients.

METHODS AND ANALYSIS

Study objectives

The PINPOINT study is designed to compare pharmacokinetics and pharmacodynamics of ticagrelor and its active metabolite (AR-C124910XX) in patients with STEMI and NSTEMI designated to invasive strategy.

Study design

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational, pharmacokinetic-pharmacodynamic study. After admission to the study centre (Cardiology Clinic, Dr. A. Jurasz University Hospital, Bydgoszcz, Poland) and confirmation of STEMI or NSTEMI diagnosis, patients will be screened for eligibility for the study. Each patient will provide a written informed consent to participate in the trial. All included patients will receive orally a 300 mg LD of plain aspirin and a 180 mg LD of ticagrelor in integral tablets with 250 mL of tap water. Subsequently, all patients will undergo a coronary angiography followed by PCI, if required. Blood samples for pharmacokinetic and pharmacodynamic assessment will be drawn at eight predefined time points according to the blood sampling schedule already used at our site in a previous study (pre-treatment baseline, 30 min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - as shown in Figure 1).[26]

Study population

The study population will include consecutive adult, male or non-pregnant female STEMI and NSTEMI patients, designated to invasive strategy. Full list of inclusion and exclusion criteria are presented in Table 1.

Table 1. Inclusion and exclusion criteria of the PINPOINT study.

Inclusion criteria	Exclusion criteria
provision of informed consent prior to any study specific procedures	treatment with ticlopidine, clopidogrel, prasugrel or ticagrelor within 14 days before the study enrolment
diagnosis of STEMI or NSTEMI	hypersensitivity to ticagrelor
male or non-pregnant female, 18 years old and older	current treatment with oral anticoagulant or chronic therapy with low-molecular-weight heparin
provision of informed consent for angiography and PCI	active bleeding
	history of intracranial haemorrhage
	recent gastrointestinal bleeding (within 30 days)
	history of coagulation disorders
	history of moderate or severe hepatic impairment
	history of major surgery or severe trauma (within 3 months)
	second or third degree atrioventricular block during screening for eligibility
	patient required dialysis
	manifest infection or inflammatory state
	Killip class III or IV during screening for eligibility
	respiratory failure
	current therapy with strong CYP3A inhibitors or strong CYP3A inducers

NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction.

Assessment of pharmacokinetics

Blood plasma concentrations of ticagrelor and AR-C124910XX in samples obtained at all eight predefined time points (Figure 1) will be evaluated using liquid chromatography mass spectrometry coupled with tandem mass spectrometry.

Assessment of pharmacodynamics

Platelet VASP assay will be applied to all study participants at all predefined time points. Multiple electrode aggregometry (MEA) will be used at all predefined time points (Figure 1) for all study participants with the exception of those treated with glycoprotein IIb/IIIa (GP IIb/IIIa) receptor inhibitors as this therapy may affect the results of platelet reactivity assessment with MEA (Figure 2).

Treatment

All patients included in the trial will be treated according to the current ESC guidelines. Standard therapy will include aspirin, ticagrelor, beta-blockers, statins, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, if not contraindicated. The type of implanted stent and the choice of the access site for the coronary invasive procedure (radial or femoral) will be at the discretion of the operator. Administration of GP IIb/IIIa receptor inhibitors will be restricted only to bailout situations. Interventional cardiologists will be encouraged to use manual thrombectomy in case of visible thrombus.

Study endpoints

The primary end-point of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor for the first 6 hours after LD of ticagrelor. Secondary end-points include $AUC_{(0–6)}$ for AR-C124910XX, area under the plasma concentration-time curve ($AUC_{(0–12)}$) for ticagrelor for the first 12 hours after LD of ticagrelor, $AUC_{(0–12)}$ for AR-C124910XX, maximum concentration (C_{max}) of ticagrelor and AR-C124910XX, time to maximum concentration (t_{max}) for ticagrelor and AR-C124910XX, platelet reactivity index (PRI) assessed by the VASP assay, platelet reactivity assessed by MEA, percentage of patients with HPR after LD of ticagrelor assessed with the VASP assay and MEA, time to reach platelet reactivity below the cut-off value for HPR evaluated with the VASP assay and MEA.

Statistical analysis

The continuous variables in the both study groups will be compared by t-test for normally distributed values as assessed by Kolmogorov-Smirnov test. Otherwise, the Mann-Whitney U test will be used. Proportions will be compared by the chi-square test when appropriate. Pharmacokinetic calculations and plots will be made using dedicated software.

Determination of sample size

Since there is no reference study comparing pharmacokinetics of ticagrelor in STEMI and NSTEMI patients, we decided to perform an internal pilot study of at least 15 patients with each type of AMI for estimating the final sample size. Eventually, the pilot study population comprised of 45 patients (15 with NSTEMI and 30 with STEMI). This included all consecutively enrolled study participants who entered the trial until the minimum planned number of patients was reached in the less numerous group (NSTEMI).

The means and standard deviations of $AUC_{(0–6)}$ for ticagrelor in the first 30 STEMI patients and 15 NSTEMI patients were 2382 ± 2282 and 6406 ± 4082 ng*h/ml, respectively. Based on these results and assuming a two-sided alpha value of 0.05, we calculated, using the t-test for independent variables, that enrolment of at least 23 patients in each study arm would provide a 95% power to demonstrate a significant difference in $AUC_{(0–6)}$ for ticagrelor between patients with different type of MI.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the principles contained in the Declaration of Helsinki and Good Clinical Practice guidelines. The study received a favourable ethical opinion and approval from the Local Ethics Committee to conduct the study (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; study approval reference number KB 617/2015). Each patient will provide a written informed consent to participate in the study.

Safety

The following safety endpoints will be recorded: all-cause death, recurrent myocardial infarction according to the Third Universal Definition of Myocardial Infarction, stroke, and transient ischemic attack according to definitions used in the PLATO trial, definite stent thrombosis according to the Academic Research Consortium criteria, minor and major bleedings according to the TIMI (thrombolysis in myocardial infarction) criteria, dyspnea adverse events according to criteria used in the PLATO trial, bradyarrhythmic events according to criteria used in the PLATO trial.

Present status

The approval of the Local Ethic Committee was obtained on September 29, 2015. On November 9, 2015 the PINPOINT study was registered on ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT02602444). The first patient was enrolled in November 2015. The baseline characteristics of patients included in the pilot study are presented in Table 2.

Table 2. Baseline characteristics of patients included in the internal pilot study.

	STEMI (n=30)	NSTEMI (n=15)
Age [years]	62.3 ± 8.8	63.9 ± 9.7
Age ≥ 70 years	6 (20.0%)	4 (26.7%)
Female	6 (20.0%)	5 (33.3%)

BMI [kg/m2]	28.6 ± 4.1	27.8 ± 4.2
Hypertension	10 (33.3%)	10 (66.7%)
Diabetes mellitus	6 (20.0%)	2 (13.3%)
Dyslipidaemia	27 (90.0%)	14 (93.3%)
Current smoker	13 (43.3%)	5 (33.3%)
Prior MI	0	2 (13.3%)
Prior PCI	2 (6.7%)	3 (20.0%)
Prior CABG	0	0
Congestive heart failure	0	0
Nonhemorrhagic stroke	0	0
Peripheral arterial disease	1 (3.3%)	2 (13.3%)
Chronic renal disease	0	0
Chronic obstructive pulmonary disease	0	0
Gout	1 (3.3%)	1 (6.7%)

BMI: body mass index; CABG: coronary artery bypass surgery; MI: myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction. Data are shown as mean ± standard deviation or number (%).

Dissemination of results

Results of the PINPOINT study will be disseminated through conference presentations and peer-reviewed journals. The results will also be made available through study record website at ClinicalTrials.gov.

SUMMARY

Is it unknown whether ticagrelor's pharmacokinetic profile and antiplatelet effect are uniform in STEMI and NSTEMI patients, who are a very heterogeneous population. The PINPOINT trail is expected to be the first study to elucidate whether diagnosis of STEMI is associated with poorer absorption and subsequently weaker antiplatelet action of ticagrelor when compared with NSTEMI patients.

Contributors

JK and PA conceived the study. JK and PA wrote the study protocol with consultation from

MO, JS, KO, KB, MKr, GS, MM and MKo. Subsequently JK, PA, MO, JS, KO, KB, MKr, GS, MM and MKo revised the manuscript critically for important intellectual content. All the authors read and approved the final manuscript.

Competing interests

Dr. Jacek Kubica received a consulting fee from AstraZeneca. Dr. Marek Koziński received honoraria for lectures from AstraZeneca. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Funding

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Ethics approval

Local Ethics Committee: Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy (study approval reference number: KB 617/2015).

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Figure 1. The PINPOINT study schema.

ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

Figure 2. Platelet reactivity evaluation schedule for the PINPOINT study.

GP IIb/IIIa: glycoprotein IIb/IIIa; MEA: multiple electrode aggregometry; VASP: vasodilator-stimulated phosphoprotein.

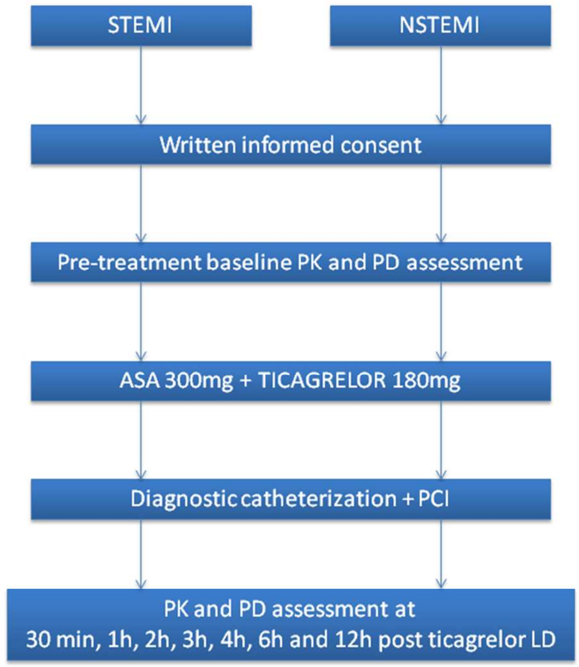

Figure 1. PINPOINT study schema.
ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

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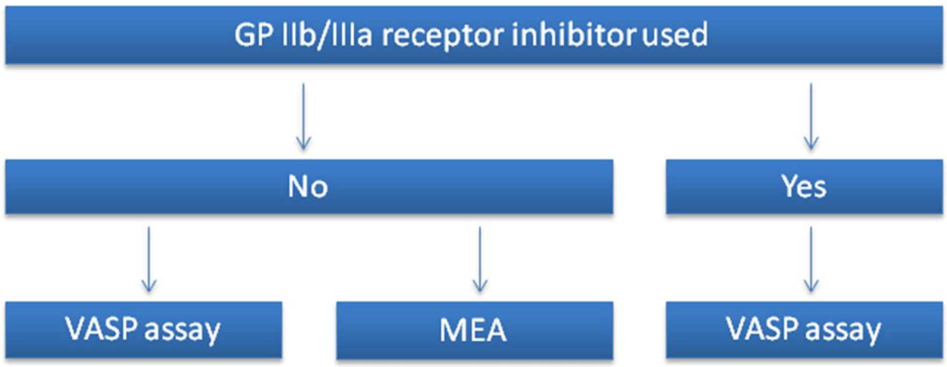

Figure 2. Platelet reactivity evaluation schedule for the PINPOINT study.
GP IIb/IIIa: glycoprotein IIb/IIIa; MEA: multiple electrode aggregometry; VASP: vasodilator-stimulated phosphoprotein.

145x60mm (120 x 120 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

Protocol for the PINPOINT study.

		Item No	Recommendation
✓	**Title and abstract**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
	Introduction		
✓	Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
✓	Objectives	3	State specific objectives, including any prespecified hypotheses
	Methods		
✓	Study design	4	Present key elements of study design early in the paper
✓	Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✓	Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
✓	Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✓	Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
	Bias	9	Describe any efforts to address potential sources of bias
✓	Study size	10	Explain how the study size was arrived at
	Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✓	Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Comment [PA1]: Will be published in the main publication with final results of the study.

Comment [PA2]: Will be published in the main publication with final results of the study.

Results			
✓ Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
✓ Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
✓ Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Comment [PA3]:** Will be published in the main publication with final results of the study.
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**Comment [PA4]:** Will be published in the main publication with final results of the study.
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Comment [PA5]:** Will be published in the main publication with final results of the study.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Comment [PA6]:** Will be published in the main publication with final results of the study.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Comment [PA7]:** Will be published in the main publication with final results of the study.
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Comment [PA8]:** Will be published in the main publication with final results of the study.
Other information			
✓ Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

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Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study

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Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study

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ABSTRACT

Introduction

The most often applied classification of acute myocardial infarction (AMI) is based on the electrocardiographic findings and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). Epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI. Ticagrelor is a P2Y12 receptor inhibitor which is the first line treatment in both STEMI and NSTEMI patients. Available data suggest that STEMI diagnosis can be associated with lower plasma concentrations of ticagrelor in the first hours of AMI, but currently there are no studies directly comparing ticagrelor's pharmacokinetics or antiplatelet effect in STEMI and NSTEMI patients.

Methods and analysis

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational study designed to compare pharmacokinetics and pharmacodynamics of ticagrelor in STEMI and NSTEMI patients designated to invasive strategy. Based on the internal pilot study, the trial is expected to include at least 23 patients with each AMI type. All subjects will receive 180 mg loading dose of ticagrelor. The primary end-point of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor for the first 6 hours after the loading dose. Secondary end-points include various pharmacokinetic features of ticagrelor and its active metabolite (AR-C124910XX), and evaluation of platelet reactivity by VASP assay and multiple electrode aggregometry. Blood samples for the pharmacokinetic and pharmacodynamic assessment will be obtained at pre-treatment, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor loading dose.

Ethics and dissemination

The study received approval from the Local Ethics Committee to conduct the study (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; approval reference number KB 617/2015). The study results will be disseminated through conference presentations and peer-reviewed journals.

Trial Registration: ClinicalTrials.gov identifier: NCT02602444 (November 09, 2015)

INTRODUCTION

Background

Third Universal Definition of Myocardial Infarction recognizes five different types of myocardial infarction based on their pathomechanism or clinical cause.[1] However, a different classification is routinely applied in everyday practice to facilitate the immediate choice of treatment strategy in patients with acute myocardial infarction (AMI). This classification is based on the electrocardiographic findings and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI).[1]

Over the past years the incidence of STEMI has decreased, while the occurrence of NSTEMI has slightly increased, and currently STEMI and NSTEMI occur almost equally often.[2, 3] Short-term mortality is higher in STEMI patients, however the mortality rates become comparable or even higher in NSTEMI patients at long-term follow-up.[4-7]

In STEMI, which is usually caused by acute total occlusion of coronary artery, immediate primary percutaneous coronary intervention (PCI) is the mainstay of treatment.[8] In NSTEMI, the therapeutic strategy and its timing depends on the risk stratification.[7] Complementary to coronary revascularization, dual antiplatelet therapy consisting of aspirin on top of a P2Y12 receptor inhibitor remains the cornerstone of pharmacological treatment in AMI patients, including both STEMI and NSTEMI.[9] The importance of platelet P2Y12 receptor blockade in patients with AMI derives from the essential role exerted by platelet activation and aggregation in the pathophysiology of acute coronary syndromes (ACS).[10] Inadequate platelet inhibition during treatment with P2Y12 receptor inhibitors, defined as high platelet reactivity (HPR), is an important risk factor for stent thrombosis and can be associated with increased mortality.[11, 12] Therefore, effective and rapid suppression of platelet activation is pivotal in patients with AMI treated with PCI.

Ticagrelor is a reversible, oral P2Y12 receptor inhibitor which is recommended as the first line treatment both in STEMI and NSTEMI patients.[13, 14] Ticagrelor is characterized by a linear pharmacokinetics and does not require hepatic metabolism to exert its antiplatelet action. Nevertheless, it is extensively metabolised by hepatic CYP3A enzymes.[15] AR-C124910XX is the major active metabolite of ticagrelor and it produces similar antiplatelet effect as the parent drug. After oral ingestion of ticagrelor, AR-C124910XX quickly appears in circulation and reaches approximately one third of ticagrelor plasma concentration.[15] The remaining 9 of identified ticagrelor metabolites appear not to be clinically significant. Noteworthy, it has been reported that platelet inhibition by ticagrelor and AR-C124910XX is proportional to their plasma concentrations.[16]

Rationale

Impact of numerous clinical features on plasma concentration and pharmacodynamics of ticagrelor has been inspected. Genetic effects, gender, age, concomitant food intake or preloading with clopidogrel have at most minimal influence on pharmacokinetics of ticagrelor, which does not translate into any clinically significant differences in the degree of platelet inhibition.[17-20] On the other hand, morphine administration has been shown to affect ticagrelor's pharmacokinetic profile as well as antiplatelet effect not only in healthy volunteers, but also in AMI patients.[21-23] Negative impact of morphine on the intestinal absorption has been proposed as an explanation for the observed interactions, while no evidence was found that morphine affects ticagrelor conversion to its active metabolite.[22, 24] Moreover, STEMI diagnosis has also been postulated to affect ticagrelor pharmacokinetics in AMI patients. Franchi et al. reported that ticagrelor exposure is attenuated and delayed not only in STEMI patients receiving morphine, but also in opioid-naive STEMI subjects.[25] This may indicate that morphine is not exclusively responsible for the observed lower concentrations of ticagrelor in STEMI patients when compared with healthy volunteers or stable coronary artery disease patients.[20, 25, 26] Additionally, multiple regression analysis of data obtained in the randomized IMPRESSION study (Influence of Morphine on Pharmacokinetics and Pharmacodynamics of Ticagrelor in Patients with Acute Myocardial Infarction) suggests that clinical presentation as STEMI when compared with NSTEMI is independently associated with lower plasma concentrations of ticagrelor.[22]

Even though ticagrelor shows more potent and prompt platelet inhibition than clopidogrel, it still fails to provide a desired antiplatelet effect in all STEMI patients during the first hours after the loading dose (LD). At 2 hours after ticagrelor LD up to 60% of STEMI patients may still suffer from inadequate platelet inhibition.[22, 25, 27] Data on the proportion of NSTEMI patients loaded with ticagrelor who are at risk of HPR during peri-PCI period is sparse, however as expected ticagrelor has been shown to provide stronger platelet blockade than clopidogrel in this clinical setting.[28] Solely pharmacodynamic study by Laine et al. reported that platelet reactivity assessed with the platelet vasodilator-stimulated phosphoprotein (VASP) assay after administration of a 180 mg ticagrelor LD was not uniform among ACS patients, but when grouped by ACS type (STEMI, NSTEMI and unstable angina) it appeared to be similar (p=0.9). However, the authors assessed the antiplatelet effect of ticagrelor only once in each patient and the time of blood sampling differed substantially among trial participants. Additionally, blood samples for pharmacodynamic evaluation were obtained between 6 and 24 hours after ticagrelor LD, leaving the first crucial hours after PCI not covered by the analysis.[29]

Although mechanistic studies are lacking, diminished plasma concentration of ticagrelor after LD observed in STEMI patients is most likely related to worse bioavailability of ticagrelor in this setting. Apart from morphine administration, other factors also may contribute to reduced gastrointestinal uptake of ticagrelor in STEMI. Adrenergic activation, decreased cardiac output, hemodynamic instability and vasoconstriction of peripheral arteries, more frequently observed in STEMI patients, lead to selective shunting of blood in order to maintain sufficient perfusion of vital organs.[30, 31] This chain of events eventually may cause intestinal hypoperfusion, which together with emesis potentially could explain poorer absorption of oral agents, including ticagrelor, in STEMI patients. Usually, NSTEMI course is less dramatic, but whether significant impairment of ticagrelor absorption with subsequent inadequate platelet blockade occurs in these patients, remain unknown.

The Platelet Inhibition and Patient Outcomes (PLATO) study has shown a remarkable reduction in cardiovascular events and all-cause mortality among ACS patients treated with ticagrelor compared with those receiving clopidogrel. This superiority was demonstrated in most of the analysed subgroups, including patients with STEMI and NSTEMI.[32] Nevertheless, epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI, while recommended dosing regimens of ticagrelor are identical in both clinical settings.[7, 8]

Currently, there are no data on direct comparison of ticagrelor's pharmacokinetics in the mentioned types of AMI, while STEMI patients may be at risk of having lower ticagrelor plasma concentrations in the most crucial time during the early hours of AMI treatment.[22] Similarly, potential differences in ticagrelor's antiplatelet action between STEMI and NSTEMI have not been defined yet. Therefore, we decided to verify whether pharmacokinetics and pharmacodynamics of ticagrelor differ between STEMI and NSTEMI patients. The Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT) study is expected to provide a valuable insight into our knowledge regarding modern treatment of AMI patients.

METHODS AND ANALYSIS

Study objectives

The PINPOINT study is designed to compare pharmacokinetics and pharmacodynamics of ticagrelor and its active metabolite (AR-C124910XX) in patients with STEMI and NSTEMI designated to invasive strategy.

Study design

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational, pharmacokinetic-pharmacodynamic study. After admission to the study centre (Cardiology Clinic, Dr. A. Jurasz University Hospital, Bydgoszcz, Poland) and confirmation of STEMI or NSTEMI diagnosis according to the Third Universal Definition of Myocardial Infarction,[1] patients will be screened for eligibility for the study. Before any study specific procedure, each patient will provide a written informed consent to participate in the trial. All included patients will immediately receive orally a 300 mg LD of plain aspirin in integral tablets and a 180 mg LD of ticagrelor in integral tablets with 250 mL of tap water. Subsequently, all patients will promptly undergo a coronary angiography followed by PCI, if required. Blood samples for pharmacokinetic and pharmacodynamic assessment will be drawn at eight predefined time points according to the blood sampling schedule already used at our site in a previous study (pre-treatment baseline, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - as shown in Figure 1).[33]

All enrolled patients with the initial AMI diagnosis not confirmed will be excluded from the primary analysis. Patients qualified for urgent CABG within the blood sampling period also will not be included in the analysis. All study participants not receiving PCI will be reported.

Study population

The study population will include consecutive adult, male or non-pregnant female, P2Y12 receptor inhibitor-naive STEMI and NSTEMI patients, designated to invasive strategy. Full list of inclusion and exclusion criteria are presented in Table 1.

Table 1. Inclusion and exclusion criteria of the PINPOINT study.

Inclusion criteria	Exclusion criteria
• provision of informed consent prior to any study specific procedure	• treatment with ticlopidine, clopidogrel, prasugrel or ticagrelor within 14 days before the study enrolment
• diagnosis of STEMI or NSTEMI	• hypersensitivity to ticagrelor
• male or non-pregnant female, 18 years old and older	• current treatment with oral anticoagulant or chronic therapy with low-molecular-weight heparin
• provision of informed consent for angiography and PCI	• active bleeding
	• history of intracranial haemorrhage
	• fibrinolytic treatment during the index event
	• recent gastrointestinal bleeding (within 30 days)
	• history of coagulation disorders
	• history of moderate or severe hepatic impairment
	• history of major surgery or severe trauma (within 3 months)
	• second or third degree atrioventricular block during screening for eligibility
	• patient required dialysis
	• manifest infection or inflammatory state
	• Killip class III or IV during screening for eligibility
	• respiratory failure
	• current therapy with strong CYP3A inhibitors or strong CYP3A inducers

NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction.

Blood sample processing

Blood samples for pharmacokinetic and pharmacodynamic evaluation will be obtained using a venous catheter (18G) inserted into a forearm vein at eight prespecified time-points (before ticagrelor LD, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - Figure 1).

Venous blood for the pharmacokinetic evaluation will be collected into lithium-heparin vacuum test-tubes. Immediately after collection, each sample will be placed on dry ice and will be transferred to the central laboratory. Subsequently, within 20 minutes from collection, the blood will be centrifuged at 1500 g for 12 minutes at 4ºC. Within 10 minutes post-centrifugation, obtained plasma samples will be stored at temperature below -60ºC until analyzed.

Venous blood for the assessment of pharmacodynamics with VASP assay and multiple electrode aggregometry (MEA) will be collected into trisodium citrate and hirudin vacuum test-tubes, respectively. The first 3-5 mL of blood will be discarded to avoid spontaneous platelet activation. Pharmacodynamic analysis will be performed for each sample within 24h and 60min from blood collection for VASP and MEA, respectively.

Assessment of pharmacokinetics

Blood plasma concentrations of ticagrelor and AR-C124910XX in samples obtained at all eight predefined time points (Figure 1) will be evaluated using liquid chromatography mass spectrometry coupled with tandem mass spectrometry, as previously described.[22, 34] Briefly, ticagrelor and AR-C124910XX will be extracted using 4ºC methanol solution containing [2H7]ticagrelor internal standard (TM-ALS-13-226-P1, ALSACHIM, France), while calibration curves will be obtained using ticagrelor (SVI-ALS-13-146, ALSACHIM, France) and AR-C124910XX (TM-ALS-13-193-P1, ALSACHIM, France) standards. Analysis will be performed using the Shimadzu UPLC Nexera X2 system consisting of LC-30AD pumps, SIL-30AC Autosampler, CTO-20AC column oven, FCV-20-AH2 valve unit, and DGU-20A5R degasser coupled with Shimadzu 8030 ESI-QqQ mass spectrometer. Lower limits of quantification are 4.69 ng/mL for both ticagrelor and AR-C124910XX.

Assessment of pharmacodynamics

Platelet VASP assay (Biocytex, Inc., Marseille, France) will be applied to all study participants at all predefined time points. MEA (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) will be used at all predefined time points (Figure 1) for all study participants with the exception of those treated with glycoprotein IIb/IIIa (GP IIb/IIIa) receptor inhibitors as this therapy may affect the results of platelet reactivity assessment with MEA (Figure 2). Pharmacodynamic assessment with VASP and MEA will be performed according to the manufacturers' instructions, as previously described.[35, 36] HPR will be defined as platelet reactivity index (PRI) >50% and area under the aggregation curve >46 units, when evaluated with VASP and MEA, respectively.[37]

Treatment

All patients included in the trial will be treated according to the current European Society of Cardiology (ESC) guidelines.[7, 8, 38] Standard therapy will include aspirin, ticagrelor, beta-blockers, statins, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, if not contraindicated. Morphine will be used at the discretion of the ambulance staff and the attending physician. The type of implanted stent and the choice of the access site for the coronary invasive procedure (radial or femoral) will be at the discretion of the operator. During the periprocedural period, all study participants will receive unfractionated heparin in body weight adjusted dose according to the ESC recommendations.[7, 8, 38] Administration of GP IIb/IIIa receptor inhibitors will be restricted only to bailout situations. Interventional cardiologists will be encouraged to use manual thrombectomy in case of visible thrombus.

Study endpoints

The primary end-point of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor for the first 6 hours after LD of ticagrelor. Secondary end-points include $AUC_{(0–6)}$ for AR-C124910XX, area under the plasma concentration-time curve

($AUC_{(0–12)}$) for ticagrelor for the first 12 hours after LD of ticagrelor, $AUC_{(0–12)}$ for AR-C124910XX, maximum concentration (C_{max}) of ticagrelor and AR-C124910XX, time to maximum concentration (t_{max}) for ticagrelor and AR-C124910XX, PRI assessed by the VASP assay, platelet reactivity assessed by MEA, percentage of patients with HPR after ticagrelor LD assessed with the VASP assay and MEA, time to reach platelet reactivity below the cut-off value for HPR evaluated with the VASP assay and MEA.

Statistical analysis

The continuous variables in the both study groups will be compared by t-test for normally distributed values as assessed by Kolmogorov-Smirnov test. Otherwise, the Mann-Whitney U test will be used. Proportions will be compared by the chi-square test when appropriate. A single linear regression analysis will be performed and will be followed by a multiple regression analysis in case any variables are found to significantly affect the study primary end-point. Pharmacokinetic calculations and plots will be made using dedicated software.

Determination of sample size

Since there is no reference study comparing pharmacokinetics of ticagrelor in STEMI and NSTEMI patients, we decided to perform an internal pilot study of at least 15 patients with each type of AMI for estimating the final sample size. Eventually, the pilot study population comprised of 45 patients (15 with NSTEMI and 30 with STEMI). This included all consecutively enrolled study participants who entered the trial until the minimum planned number of patients was reached in the less numerous group (NSTEMI).

The means and standard deviations of $AUC_{(0–6)}$ for ticagrelor in the first 30 STEMI patients and 15 NSTEMI patients were 2382 ± 2282 and 6406 ± 4082 ng*h/ml, respectively. Based on these results and assuming a two-sided alpha value of 0.05, we calculated, using the t-test for independent variables, that enrolment of at least 23 patients in each study arm would provide a 95% power to demonstrate a significant difference in $AUC_{(0–6)}$ for ticagrelor between patients with different type of MI.

Study limitations

Several limitations of our study have to be acknowledged. First, the anticipated trial population will not be sufficient to evaluate clinical end-points and most likely to perform subgroup analyses. Second, patients receiving morphine are not excluded from the study, which may result in the baseline characteristics differences between the examined groups. Third, morphine is used at the discretion of the paramedics or the attending physicians, although we encourage the medical staff to administer a standardized dose of 5 mg intravenously, if required in any potential or actual study participant. On the other hand, even though it may be perceived as a limitation, this will enable us to obtain a real life data and will not create artificially selected population.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the principles contained in the Declaration of Helsinki and Good Clinical Practice guidelines. The study received a favourable ethical opinion and approval from the Local Ethics Committee to conduct the study (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; study approval reference number KB 617/2015). Each patient will provide a written informed consent to participate in the study.

Safety

The following safety endpoints will be recorded during the blood sampling period: all-cause death, recurrent myocardial infarction according to the Third Universal Definition of Myocardial Infarction, stroke, and transient ischaemic attack according to definitions used in the PLATO trial, definite or probable stent thrombosis according to the Academic Research Consortium criteria, minor and major bleedings according to the TIMI (thrombolysis in myocardial infarction) criteria, dyspnea adverse events according to criteria used in the PLATO trial, bradyarrhythmic events according to criteria used in the PLATO trial.

Present status

The approval of the Local Ethic Committee was obtained on September 29, 2015. On November 9, 2015 the PINPOINT study was registered on ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT02602444). The first patient was enrolled in November 2015. The baseline characteristics of patients included in the pilot study are presented in Table 2.

Table 2. Baseline characteristics of patients included in the internal pilot study.

	STEMI (n=30)	NSTEMI (n=15)	p value
Age [years]	62.3 ± 8.8	63.9 ± 9.7	0.51
Age ≥70 years	6 (20.0%)	4 (26.7%)	0.89
Female	6 (20.0%)	5 (33.3%)	0.53
BMI [kg/m2]	28.6 ± 4.1	27.8 ± 4.2	0.76
Hypertension	10 (33.3%)	10 (66.7%)	0.036
Diabetes mellitus	6 (20.0%)	2 (13.3%)	0.89
Dyslipidaemia	27 (90.0%)	14 (93.3%)	0.85
Current smoker	13 (43.3%)	5 (33.3%)	0.52
Prior MI	0	2 (13.3%)	n/a
Prior PCI	2 (6.7%)	3 (20.0%)	0.4
Prior CABG	0	0	n/a
Congestive heart failure	0	0	n/a
Nonhaemorrhagic stroke	0	0	n/a
Peripheral arterial disease	1 (3.3%)	2 (13.3%)	0.21
Chronic renal disease	0	0	n/a
Chronic obstructive pulmonary	0	0	n/a

disease			
Gout	1 (3.3%)	1 (6.7%)	n/a
Morphine use during current MI	17 (56.7%)	6 (40.0%)	0.29

BMI: body mass index; CABG: coronary artery bypass surgery; MI: myocardial infarction; n/a: not available; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction. Data are shown as mean ± standard deviation or number (%).

Dissemination of results

Results of the PINPOINT study will be disseminated through conference presentations and peer-reviewed journals. The results will also be made available through study record website at ClinicalTrials.gov.

SUMMARY

Is it unknown whether ticagrelor's pharmacokinetic profile and antiplatelet effects are uniform in STEMI and NSTEMI patients, who are a very heterogeneous population. The PINPOINT trial is expected to be the first study to elucidate whether diagnosis of STEMI is associated with poorer absorption and subsequently weaker antiplatelet action of ticagrelor when compared with NSTEMI patients.

Contributors

JK and PA conceived the study. JK and PA wrote the study protocol with consultation from MO, JS, KO, KB, MKr, GS, MM and MKo. Subsequently JK, PA, MO, JS, KO, KB, MKr, GS, MM and MKo revised the manuscript critically for important intellectual content. All the authors read and approved the final manuscript.

Competing interests

Dr. Jacek Kubica received a consulting fee from AstraZeneca. Dr. Marek Koziński received honoraria for lectures from AstraZeneca. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Ethics approval

Local Ethics Committee: Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy (study approval reference number: KB 617/2015).

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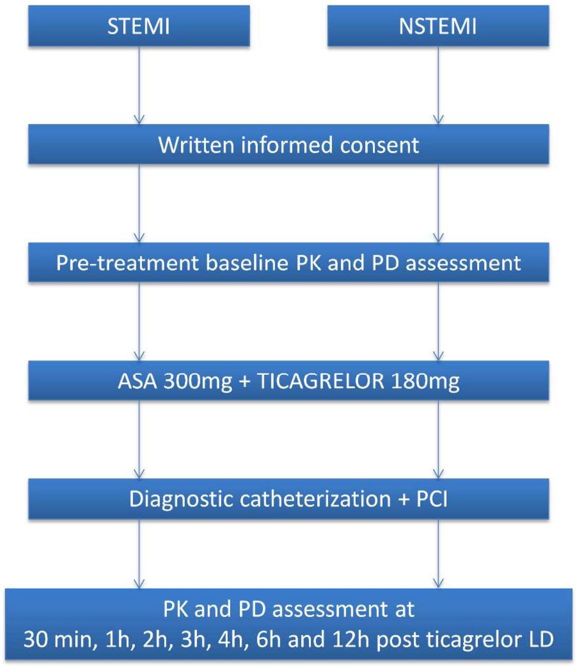
Figure 1. The PINPOINT study schema.

ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

Figure 2. Platelet reactivity evaluation schedule for the PINPOINT study.

GP IIb/IIIa: glycoprotein IIb/IIIa; MEA: multiple electrode aggregometry; VASP: vasodilator-stimulated phosphoprotein.



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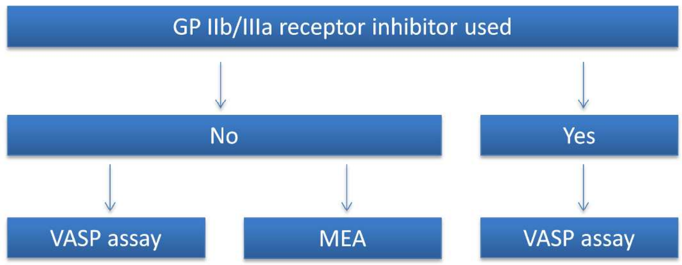

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STROBE Statement—checklist of items that should be included in reports of observational studies

Protocol for the PINPOINT study.

		Item No	Recommendation
✓	**Title and abstract**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
	Introduction		
✓	Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
✓	Objectives	3	State specific objectives, including any prespecified hypotheses
	Methods		
✓	Study design	4	Present key elements of study design early in the paper
✓	Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✓	Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
✓	Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✓	Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
	Bias	9	Describe any efforts to address potential sources of bias
✓	Study size	10	Explain how the study size was arrived at
	Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✓	Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Comment [PA1]: Will be published in the main publication with final results of the study.

Comment [PA2]: Will be published in the main publication with final results of the study.

Results			
✓ Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
✓ Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
✓ Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Comment [PA3]:** Will be published in the main publication with final results of the study.
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**Comment [PA4]:** Will be published in the main publication with final results of the study.
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Comment [PA5]:** Will be published in the main publication with final results of the study.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Comment [PA6]:** Will be published in the main publication with final results of the study.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Comment [PA7]:** Will be published in the main publication with final results of the study.
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Comment [PA8]:** Will be published in the main publication with final results of the study.
Other information			
✓ Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

BMJ Open

Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study

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Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	NSTEMI, pharmacodynamics, pharmacokinetics, STEMI, ticagrelor

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Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study

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ABSTRACT

Introduction

The most often applied classification of acute myocardial infarction (AMI) is based on the electrocardiographic findings and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). Epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI. Ticagrelor is a P2Y12 receptor inhibitor which is the first line treatment in both STEMI and NSTEMI patients. Available data suggest that STEMI diagnosis can be associated with lower plasma concentrations of ticagrelor in the first hours of AMI, but currently there are no studies directly comparing ticagrelor's pharmacokinetics or antiplatelet effect in STEMI and NSTEMI patients.

Methods and analysis

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational study designed to compare pharmacokinetics and pharmacodynamics of ticagrelor in STEMI and NSTEMI patients designated to invasive strategy. Based on the internal pilot study, the trial is expected to include at least 23 patients with each AMI type. All subjects will receive 180 mg loading dose of ticagrelor. The primary end-point of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor for the first 6 hours after the loading dose. Secondary end-points include various pharmacokinetic features of ticagrelor and its active metabolite (AR-C124910XX), and evaluation of platelet reactivity by VASP assay and multiple electrode aggregometry. Blood samples for the pharmacokinetic and pharmacodynamic assessment will be obtained at pre-treatment, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor loading dose.

Ethics and dissemination

The study received approval from the Local Ethics Committee to conduct the study (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; approval reference number KB 617/2015). The study results will be disseminated through conference presentations and peer-reviewed journals.

Trial Registration: ClinicalTrials.gov identifier: NCT02602444 (November 09, 2015)

STRENGHTS AND LIMITATIONS

- This is the first study to provide prospective head-to-head comparison of ticagrelor pharmacokinetics and pharmacodynamics between STEMI and NSTEMI patients designated to invasive strategy.
- Plasma concentrations of ticagrelor and its active metabolite will be assessed with liquid chromatography mass spectrometry coupled with tandem mass spectrometry.
- Antiplatelet effect of ticagrelor will be evaluated with two commonly recognized methods: vasodilator-stimulated phosphoprotein (VASP) assay and multiple electrode aggregometry.
- This is purely pharmacokinetic/pharmacodynamic study, thus unfortunately the anticipated trial population most likely will not be sufficient to evaluate clinical end-points or to perform subgroup analyses.
- Patients receiving morphine are not excluded from the study, which may result in the baseline characteristics differences between the examined groups, but this will enable us to obtain a real life data and will not create artificially selected population.

INTRODUCTION

Background

Classification of acute myocardial infarction (AMI) routinely applied in everyday practice to facilitate the choice of treatment strategy is based on the electrocardiographic findings, and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI).[1]

In STEMI, which is usually caused by acute total occlusion of coronary artery, immediate primary percutaneous coronary intervention (PCI) is the mainstay of treatment.[2] In NSTEMI, the therapeutic strategy and its timing depends on the risk stratification.[3] Complementary to coronary revascularization, dual antiplatelet therapy consisting of aspirin on top of a P2Y12 receptor inhibitor remains the cornerstone of pharmacological treatment in AMI patients, including both STEMI and NSTEMI.[4, 5] Inadequate platelet inhibition during treatment with P2Y12 receptor inhibitors, defined as high platelet reactivity (HPR), is an important risk factor for stent thrombosis and can be associated with increased mortality.[6, 7] Therefore, effective and rapid suppression of platelet activation is pivotal in patients with AMI treated with PCI.

Ticagrelor is a reversible, oral P2Y12 receptor inhibitor which is recommended as the first line treatment both in STEMI and NSTEMI patients.[8, 9] Ticagrelor is characterized by a linear pharmacokinetics and does not require hepatic metabolism to exert its antiplatelet action. Nevertheless, it is extensively metabolised by hepatic CYP3A enzymes.[10] AR-C124910XX is the major active metabolite of ticagrelor and it produces similar antiplatelet effect as the parent drug. After oral ingestion of ticagrelor, AR-C124910XX quickly appears in circulation and reaches approximately one third of ticagrelor plasma concentration.[10] The remaining 9 of identified ticagrelor metabolites appear not to be clinically significant.

Noteworthy, it has been reported that platelet inhibition by ticagrelor and AR-C124910XX is proportional to their plasma concentrations.[11]

Rationale

Impact of numerous clinical features on plasma concentration and pharmacodynamics of ticagrelor has been inspected. Genetic effects, gender, age, concomitant food intake or preloading with clopidogrel have at most minimal influence on pharmacokinetics of ticagrelor, which does not translate into any clinically significant differences in the degree of platelet inhibition.[12-15] On the other hand, morphine administration has been shown to affect ticagrelor's pharmacokinetic profile as well as antiplatelet effect not only in healthy volunteers, but also in AMI patients.[16-18] Negative impact of morphine on the intestinal absorption has been proposed as an explanation for the observed interactions, while no evidence was found that morphine affects ticagrelor conversion to its active metabolite.[18, 19] Importantly, STEMI diagnosis has also been postulated to affect ticagrelor pharmacokinetics in AMI patients. Franchi et al. reported that ticagrelor exposure is attenuated and delayed not only in STEMI patients receiving morphine, but also in opioid-naive STEMI subjects.[20] This may indicate that morphine is not exclusively responsible for the observed lower concentrations of ticagrelor in STEMI patients when compared with healthy volunteers or stable coronary artery disease patients.[15, 20, 21] Moreover, sub-analyses of two pharmacokinetic/pharmacodynamic trials suggest that clinical presentation as STEMI when compared with NSTEMI is independently associated with lower plasma concentrations of ticagrelor.[18, 22]

Although mechanistic studies are lacking, diminished plasma concentration of ticagrelor after loading dose (LD) observed in STEMI patients is most likely related to worse bioavailability of ticagrelor in this setting. Adrenergic activation, decreased cardiac output, hemodynamic instability and vasoconstriction of peripheral arteries, more frequently observed in STEMI patients, lead to selective shunting of blood in order to maintain sufficient perfusion of vital organs.[23, 24] This chain of events eventually may cause intestinal hypoperfusion, which together with emesis potentially could explain poorer absorption of oral agents, including ticagrelor, in STEMI patients. Usually, NSTEMI course is less dramatic, but whether significant impairment of ticagrelor absorption occurs in these patients, remains unknown.

Even though ticagrelor shows potent and prompt platelet inhibition, it still fails to provide a desired antiplatelet effect in all STEMI patients during the first hours after the LD. At 2 hours after ticagrelor LD up to 60% of STEMI patients may still suffer from inadequate platelet inhibition.[18, 20, 25] Data on the proportion of NSTEMI patients loaded with ticagrelor who are at risk of HPR during peri-PCI period is sparse.

The Platelet Inhibition and Patient Outcomes (PLATO) study has shown a remarkable reduction in cardiovascular events and all-cause mortality among ACS patients treated with ticagrelor compared with those receiving clopidogrel. This superiority was demonstrated in most of the analysed subgroups, including patients with STEMI and NSTEMI.[26] Nevertheless, epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI, while recommended dosing regimens of ticagrelor are identical in both clinical settings.[2, 3, 27-30]

Currently, there are no data on direct comparison of ticagrelor's pharmacokinetics in the mentioned types of AMI, while STEMI patients may be at risk of having lower ticagrelor plasma concentrations in the most crucial time during the early hours of AMI treatment.[18, 22] Similarly, potential differences in ticagrelor's antiplatelet action between STEMI and NSTEMI have not been defined yet. Therefore, we decided to verify whether pharmacokinetics and pharmacodynamics of ticagrelor differ between STEMI and NSTEMI patients. The Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT) study is expected to provide a valuable insight into our knowledge regarding modern treatment of AMI patients.

METHODS AND ANALYSIS

Study objectives

The PINPOINT study is designed to compare pharmacokinetics and pharmacodynamics of ticagrelor and its active metabolite (AR-C124910XX) in patients with STEMI and NSTEMI designated to invasive strategy.

Study design

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational, pharmacokinetic-pharmacodynamic study. After admission to the study centre (Cardiology Clinic, Dr. A. Jurasz University Hospital, Bydgoszcz, Poland) and confirmation of STEMI or NSTEMI diagnosis according to the Third Universal Definition of Myocardial Infarction,[1] patients will be screened for eligibility for the study. Before any study specific procedure, each patient will provide a written informed consent to participate in the trial. All included patients will immediately receive orally a 300 mg LD of plain aspirin in integral tablets and a 180 mg LD of ticagrelor in integral tablets with 250 mL of tap water. Subsequently, all patients will promptly undergo a coronary angiography followed by PCI, if required. Blood samples for pharmacokinetic and pharmacodynamic assessment will be drawn at eight predefined time points according to the blood sampling schedule already used at our site in a previous study (pre-treatment baseline, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - as shown in Figure 1).[31]

All enrolled patients with the initial AMI diagnosis not confirmed will be excluded from the primary analysis. Patients qualified for urgent CABG within the blood sampling period also will not be included in the analysis. All study participants not receiving PCI will be reported.

Study population

The study population will include consecutive adult, male or non-pregnant female, P2Y12 receptor inhibitor-naive STEMI and NSTEMI patients, designated to invasive strategy. Full list of inclusion and exclusion criteria are presented in Table 1.

Table 1. Inclusion and exclusion criteria of the PINPOINT study.

Inclusion criteria	Exclusion criteria
• provision of informed consent prior to any study specific procedure	• treatment with ticlopidine, clopidogrel, prasugrel or ticagrelor within 14 days before the study enrolment
• diagnosis of STEMI or NSTEMI	• hypersensitivity to ticagrelor
• male or non-pregnant female, 18 years old and older	• current treatment with oral anticoagulant or chronic therapy with low-molecular-weight heparin
• provision of informed consent for angiography and PCI	• active bleeding
	• history of intracranial haemorrhage
	• fibrinolytic treatment during the index event
	• recent gastrointestinal bleeding (within 30 days)
	• history of coagulation disorders
	• history of moderate or severe hepatic impairment
	• history of major surgery or severe trauma (within 3 months)
	• second or third degree atrioventricular block during screening for eligibility
	• patient required dialysis
	• manifest infection or inflammatory state
	• Killip class III or IV during screening for eligibility
	• respiratory failure

- current therapy with strong CYP3A inhibitors or strong CYP3A inducers

NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction.

Blood sample processing

Blood samples for pharmacokinetic and pharmacodynamic evaluation will be obtained using a venous catheter (18G) inserted into a forearm vein at eight prespecified time-points (before ticagrelor LD, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - Figure 1).

Venous blood for the pharmacokinetic evaluation will be collected into lithium-heparin vacuum test-tubes. Immediately after collection, each sample will be placed on dry ice and will be transferred to the central laboratory. Subsequently, within 20 minutes from collection, the blood will be centrifuged at 1500 g for 12 minutes at 4ºC. Within 10 minutes post-centrifugation, obtained plasma samples will be stored at temperature below -60ºC until analyzed.

Venous blood for the assessment of pharmacodynamics with VASP assay and multiple electrode aggregometry (MEA) will be collected into trisodium citrate and hirudin vacuum test-tubes, respectively. The first 3-5 mL of blood will be discarded to avoid spontaneous platelet activation. Pharmacodynamic analysis will be performed for each sample within 24h and 60min from blood collection for VASP and MEA, respectively.

Assessment of pharmacokinetics

Blood plasma concentrations of ticagrelor and AR-C124910XX in samples obtained at all eight predefined time points (Figure 1) will be evaluated using liquid chromatography mass spectrometry coupled with tandem mass spectrometry, as previously described.[18, 32] Briefly, ticagrelor and AR-C124910XX will be extracted using 4ºC methanol solution containing [2H7]ticagrelor internal standard (TM-ALS-13-226-P1, ALSACHIM, France), while calibration curves will be obtained using ticagrelor (SVI-ALS-13-146, ALSACHIM, France) and AR-C124910XX (TM-ALS-13-193-P1, ALSACHIM, France) standards. Analysis will be performed using the Shimadzu UPLC Nexera X2 system consisting of LC-30AD pumps, SIL-30AC Autosampler, CTO-20AC column oven, FCV-20-AH2 valve unit, and DGU-20A5R degasser coupled with Shimadzu 8030 ESI-QqQ mass spectrometer. Lower limits of quantification are 4.69 ng/mL for both ticagrelor and AR-C124910XX.

Assessment of pharmacodynamics

Platelet VASP assay (Biocytex, Inc., Marseille, France) will be applied to all study participants at all predefined time points. MEA (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) will be used at all predefined time points (Figure 1) for all study participants with the exception of those treated with glycoprotein IIb/IIIa (GP IIb/IIIa) receptor inhibitors as this therapy may affect the results of platelet reactivity assessment with MEA (Figure 2). Pharmacodynamic assessment with VASP and MEA will be performed according to the manufacturers' instructions, as previously described.[33, 34] HPR will be defined as platelet reactivity index (PRI) >50% and area under the aggregation curve >46 units, when evaluated with VASP and MEA, respectively.[35]

Treatment

All patients included in the trial will be treated according to the current European Society of Cardiology (ESC) guidelines.[2, 3, 36] Standard therapy will include aspirin, ticagrelor, beta-blockers, statins, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, if not contraindicated. Morphine will be used at the discretion of the ambulance staff and the attending physician. The type of implanted stent and the choice of the access site for the coronary invasive procedure (radial or femoral) will be at the discretion of the operator. During the periprocedural period, all study participants will receive unfractionated heparin in body weight adjusted dose according to the ESC recommendations.[2, 3, 36] Administration of GP IIb/IIIa receptor inhibitors will be restricted only to bailout situations. Interventional cardiologists will be encouraged to use manual thrombectomy in case of visible thrombus.

Study endpoints

The primary end-point of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor for the first 6 hours after LD of ticagrelor. Secondary end-points include $AUC_{(0–6)}$ for AR-C124910XX, area under the plasma concentration-time curve ($AUC_{(0–12)}$) for ticagrelor for the first 12 hours after LD of ticagrelor, $AUC_{(0–12)}$ for AR-C124910XX, maximum concentration (C_{max}) of ticagrelor and AR-C124910XX, time to maximum concentration (t_{max}) for ticagrelor and AR-C124910XX, PRI assessed by the VASP assay, platelet reactivity assessed by MEA, percentage of patients with HPR after ticagrelor LD assessed with the VASP assay and MEA, time to reach platelet reactivity below the cut-off value for HPR evaluated with the VASP assay and MEA.

Statistical analysis

The continuous variables in the both study groups will be compared by t-test for normally distributed values as assessed by Kolmogorov-Smirnov test. Otherwise, the Mann-Whitney U test will be used. Proportions will be compared by the chi-square test when appropriate. A single linear regression analysis will be performed and will be followed by a multiple regression analysis in case any variables are found to significantly affect the study primary end-point. Pharmacokinetic calculations and plots will be made using dedicated software.

Determination of sample size

Since there is no reference study comparing pharmacokinetics of ticagrelor in STEMI and NSTEMI patients, we decided to perform an internal pilot study of at least 15 patients with each type of AMI for estimating the final sample size. Eventually, the pilot study population comprised of 45 patients (15 with NSTEMI and 30 with STEMI). This included all consecutively enrolled study participants who entered the trial until the minimum planned number of patients was reached in the less numerous group (NSTEMI).

The means and standard deviations of $AUC_{(0–6)}$ for ticagrelor in the first 30 STEMI patients and 15 NSTEMI patients were 2382 ± 2282 and 6406 ± 4082 ng*h/ml, respectively. Based on these results and assuming a two-sided alpha value of 0.05, we calculated, using the t-test for independent variables, that enrolment of at least 23 patients in each study arm would provide a 95% power to demonstrate a significant difference in $AUC_{(0–6)}$ for ticagrelor between patients with different type of MI.

Study limitations

Several limitations of our study have to be acknowledged. First, the anticipated trial population will not be sufficient to evaluate clinical end-points and most likely to perform subgroup analyses. Second, patients receiving morphine are not excluded from the study, which may result in the baseline characteristics differences between the examined groups. Third, morphine is used at the discretion of the paramedics or the attending physicians, although we encourage the medical staff to administer a standardized dose of 5 mg intravenously, if required in any potential or actual study participant. On the other hand, even though it may be perceived as a limitation, this will enable us to obtain a real life data and will not create artificially selected population.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the principles contained in the Declaration of Helsinki and Good Clinical Practice guidelines. The study received a favourable ethical opinion and approval from the Local Ethics Committee to conduct the study (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; study approval reference number KB 617/2015). Each patient will provide a written informed consent to participate in the study.

Safety

The following safety endpoints will be recorded during the blood sampling period: all-cause death, recurrent myocardial infarction according to the Third Universal Definition of Myocardial Infarction, stroke, and transient ischaemic attack according to definitions used in the PLATO trial, definite or probable stent thrombosis according to the Academic Research Consortium criteria, minor and major bleedings according to the TIMI (thrombolysis in myocardial infarction) criteria, dyspnea adverse events according to criteria used in the PLATO trial, bradyarrhythmic events according to criteria used in the PLATO trial.

Present status

The approval of the Local Ethic Committee was obtained on September 29, 2015. On November 9, 2015 the PINPOINT study was registered on ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT02602444). The first patient was enrolled in November 2015. The baseline characteristics of patients included in the pilot study are presented in Table 2.

Table 2. Baseline characteristics of patients included in the internal pilot study.

	STEMI (n=30)	NSTEMI (n=15)	p value
Age [years]	62.3 ± 8.8	63.9 ± 9.7	0.51
Age ≥70 years	6 (20.0%)	4 (26.7%)	0.89
Female	6 (20.0%)	5 (33.3%)	0.53
BMI [kg/m2]	28.6 ± 4.1	27.8 ± 4.2	0.76
Hypertension	10 (33.3%)	10 (66.7%)	0.036
Diabetes mellitus	6 (20.0%)	2 (13.3%)	0.89

Dyslipidaemia	27 (90.0%)	14 (93.3%)	0.85
Current smoker	13 (43.3%)	5 (33.3%)	0.52
Prior MI	0	2 (13.3%)	n/a
Prior PCI	2 (6.7%)	3 (20.0%)	0.4
Prior CABG	0	0	n/a
Congestive heart failure	0	0	n/a
Nonhaemorrhagic stroke	0	0	n/a
Peripheral arterial disease	1 (3.3%)	2 (13.3%)	0.21
Chronic renal disease	0	0	n/a
Chronic obstructive pulmonary disease	0	0	n/a
Gout	1 (3.3%)	1 (6.7%)	n/a
Morphine use during current MI	17 (56.7%)	6 (40.0%)	0.29

BMI: body mass index; CABG: coronary artery bypass surgery; MI: myocardial infarction; n/a: not available; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction. Data are shown as mean ± standard deviation or number (%).

Dissemination of results

Results of the PINPOINT study will be disseminated through conference presentations and peer-reviewed journals. The results will also be made available through study record website at ClinicalTrials.gov.

SUMMARY

Is it unknown whether ticagrelor's pharmacokinetic profile and antiplatelet effects are uniform in STEMI and NSTEMI patients, who are a very heterogeneous population. The PINPOINT trial is expected to be the first study to elucidate whether diagnosis of STEMI is associated with poorer absorption and subsequently weaker antiplatelet action of ticagrelor when compared with NSTEMI patients.

Contributors

JK and PA conceived the study. JK and PA wrote the study protocol with consultation from MO, JS, KO, KB, MKr, GS, MM and MKo. Subsequently JK, PA, MO, JS, KO, KB, MKr, GS, MM and MKo revised the manuscript critically for important intellectual content. All the authors read and approved the final manuscript.

Competing interests

Dr. Jacek Kubica received a consulting fee from AstraZeneca. Dr. Marek Koziński received honoraria for lectures from AstraZeneca. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Funding

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Ethics approval

Local Ethics Committee: Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy (study approval reference number: KB 617/2015).

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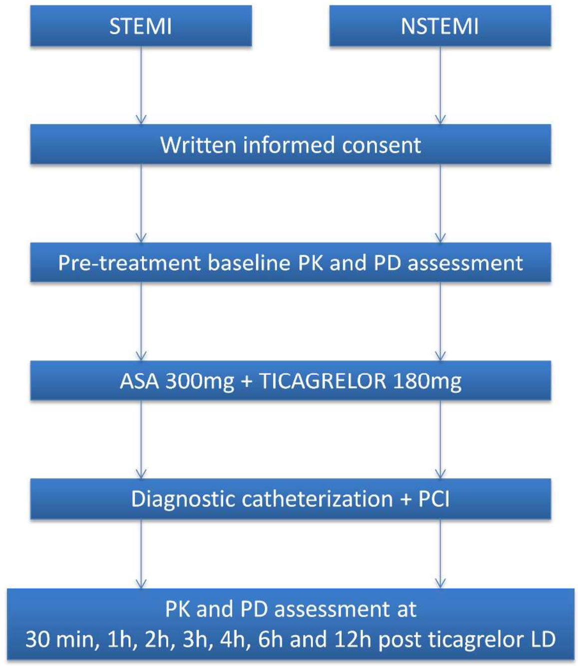
Figure 1. The PINPOINT study schema.

ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

Figure 2. Platelet reactivity evaluation schedule for the PINPOINT study.

GP IIb/IIIa: glycoprotein IIb/IIIa; MEA: multiple electrode aggregometry; VASP: vasodilator-stimulated phosphoprotein.



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ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

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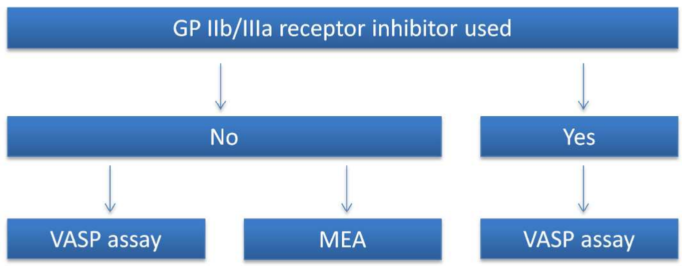

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254x190mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

Protocol for the PINPOINT study.

		Item No	Recommendation
✓	**Title and abstract**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
	Introduction		
✓	Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
✓	Objectives	3	State specific objectives, including any prespecified hypotheses
	Methods		
✓	Study design	4	Present key elements of study design early in the paper
✓	Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✓	Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
✓	Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✓	Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias		9	Describe any efforts to address potential sources of bias
✓	Study size	10	Explain how the study size was arrived at
Quantitative variables		11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✓	Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Comment [PA1]: Will be published in the main publication with final results of the study.

Comment [PA2]: Will be published in the main publication with final results of the study.

Results			
✓ Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
✓ Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
✓ Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
✓ Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

Comment [PA3]: Will be published in the main publication with final results of the study.

Comment [PA4]: Will be published in the main publication with final results of the study.

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Comment [PA7]: Will be published in the main publication with final results of the study.

Comment [PA8]: Will be published in the main publication with final results of the study.

BMJ Open

Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	NSTEMI, pharmacodynamics, pharmacokinetics, STEMI, ticagrelor

Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study

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Keywords: NSTEMI, pharmacodynamics, pharmacokinetics, STEMI, ticagrelor

Word count: 3105

ABSTRACT

Introduction

The most common classification of acute myocardial infarction (AMI) is based on electrocardiographic findings and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI). Both types of AMI differ concerning their epidemiology, clinical approach and early outcomes. Ticagrelor is a P2Y12 receptor inhibitor, constituting the first line treatment for STEMI and NSTEMI. According to available data, STEMI may be associated with lower plasma concentration of ticagrelor in the first hours of AMI, but currently there are no studies directly comparing ticagrelor pharmacokinetics or antiplatelet effect in STEMI versus NSTEMI patients.

Methods and analysis

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational study designed to compare the pharmacokinetics and pharmacodynamics of ticagrelor in STEMI and NSTEMI patients assigned to the invasive strategy of treatment. Based on an internal pilot study, the trial is expected to include at least 23 patients with each AMI type. All subjects will receive a 180 mg loading dose of ticagrelor. The primary endpoint of the study is the area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor during the first 6 hours after the loading dose. Secondary endpoints include various pharmacokinetic features of ticagrelor and its active metabolite (AR-C124910XX), and evaluation of platelet reactivity by the VASP assay and multiple electrode aggregometry. Blood samples for the pharmacokinetic and pharmacodynamic assessment will be obtained at pre-treatment, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor loading dose.

Ethics and dissemination

The study received approval from the Local Ethics Committee (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; approval reference number KB 617/2015). The study results will be disseminated through conference presentations and peer-reviewed journals.

Trial Registration: ClinicalTrials.gov identifier: NCT02602444 (November 09, 2015)

STRENGTHS AND LIMITATIONS

- This is the first study to provide prospective head-to-head comparison of ticagrelor pharmacokinetics and pharmacodynamics in STEMI versus NSTEMI patients assigned to the invasive strategy.
- Plasma concentrations of ticagrelor and its active metabolite will be assessed with liquid chromatography mass spectrometry coupled with tandem mass spectrometry.
- The antiplatelet effect of ticagrelor will be evaluated with two commonly recognized methods: the vasodilator-stimulated phosphoprotein (VASP) assay and multiple electrode aggregometry.
- As this is a purely pharmacokinetic/pharmacodynamic study, it is likely that the anticipated trial population will not be sufficient to evaluate clinical endpoints or perform subgroup analyses.
- Patients receiving morphine are not excluded from the study, which may result in differences in the baseline characteristics between the examined groups, but this will enable us to obtain data in a real-world setting and will not create an artificially selected population.

INTRODUCTION

Background

The routine classification of acute myocardial infarction (AMI) applied in everyday practice to facilitate the choice of treatment strategy is based on electrocardiographic findings, and distinguishes ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI).[1]

In STEMI, usually caused by acute total occlusion of a coronary artery, immediate primary percutaneous coronary intervention (PCI) is the mainstay of treatment.[2] In contrast to STEMI, the therapeutic strategy for NSTEMI and its timing depend on the risk stratification.[3] Complementary to coronary revascularization, dual antiplatelet therapy, consisting of aspirin on top of a P2Y12 receptor inhibitor, remains the cornerstone of pharmacological treatment in both forms of AMI.[4, 5] Inadequate platelet inhibition during treatment with P2Y12 receptor inhibitors, defined as high platelet reactivity (HPR), is an important risk factor for stent thrombosis and may be associated with increased mortality.[6, 7] Therefore, effective and rapid suppression of platelet activation is pivotal in AMI patients treated with PCI.

Ticagrelor is a reversible, oral P2Y12 receptor inhibitor, recommended as the first line treatment for STEMI and NSTEMI.[8, 9] It is characterized by linear pharmacokinetics and does not require hepatic metabolism to exert its antiplatelet action. Nevertheless, it is extensively metabolised by hepatic CYP3A enzymes.[10] AR-C124910XX is the major active metabolite of ticagrelor and it produces similar antiplatelet effect as the parent drug. After oral ingestion of ticagrelor, AR-C124910XX quickly appears in the circulation and reaches approximately one third of ticagrelor plasma concentration.[10] The remaining 9 of identified ticagrelor metabolites appear to be clinically insignificant. Ticagrelor- and AR-C124910XX-induced platelet inhibition is proportional to their plasma concentrations.[11]

Rationale

Impact of numerous clinical features on plasma concentration and pharmacodynamics of ticagrelor has been inspected. Genetic effects, gender, age, concomitant food intake or preloading with clopidogrel have at most minimal influence on the pharmacokinetics of ticagrelor and no clinically significant differences in the degree of platelet inhibition have been reported regarding these factors.[12-15] On the other hand, morphine administration has been shown to affect ticagrelor pharmacokinetic profile, as well as its antiplatelet effect, not only in healthy volunteers, but also in AMI patients.[16-18] The negative impact of morphine on the intestinal absorption has been proposed as an explanation for the observed interactions, while no evidence was found in support of the influence of morphine on conversion of ticagrelor into its active metabolite.[18, 19] Importantly, STEMI, as opposed to NSTEMI, has also been postulated to affect ticagrelor pharmacokinetics. Franchi et al. reported that ticagrelor exposure is attenuated and delayed not only in STEMI patients receiving morphine, but also in opioid-naive STEMI subjects.[20] This may indicate that morphine is not exclusively responsible for the lower concentration of ticagrelor observed in STEMI patients when compared with healthy volunteers or stable coronary artery disease patients.[15, 20, 21] Moreover, sub-analyses of two pharmacokinetic/pharmacodynamic trials suggest that STEMI in comparison with NSTEMI is independently associated with lower plasma concentration of ticagrelor.[18, 22]

Although mechanistic studies are lacking, diminished plasma concentration of ticagrelor after the loading dose (LD) observed in STEMI patients is most likely related to impaired bioavailability of ticagrelor in this setting. Adrenergic activation, decreased cardiac output, haemodynamic instability and vasoconstriction of peripheral arteries, more frequently observed in STEMI patients, lead to selective shunting of blood flow in order to maintain sufficient perfusion of vital organs.[23, 24] This chain of events eventually may cause intestinal hypoperfusion, which together with emesis could potentially explain the poorer absorption of oral agents, including ticagrelor, seen in STEMI patients. The course of NSTEMI is usually less dramatic, but it remains unknown whether significant impairment of ticagrelor absorption occurs in these patients.

Even though ticagrelor shows potent and prompt platelet inhibition, it still fails to provide a desired antiplatelet effect during the first hours after the LD in all STEMI patients. At 2 hours after ticagrelor LD up to 60% of STEMI patients may still suffer from inadequate platelet inhibition.[18, 20, 25] Data on the proportion of NSTEMI patients loaded with ticagrelor who remain at risk of HPR during the peri-PCI period is sparse.

The Platelet Inhibition and Patient Outcomes (PLATO) study has shown a remarkable reduction in cardiovascular events and all-cause mortality among acute coronary syndrome patients treated with ticagrelor compared with those receiving clopidogrel. This superiority was demonstrated in most of the analysed subgroups, including patients with STEMI and NSTEMI.[26] Nevertheless, epidemiology, clinical approach and early outcomes differ between patients with these two types of AMI, while recommended dosing regimens of ticagrelor are identical in both clinical settings.[2, 3, 27-30]

Currently, there are no data directly comparing ticagrelor pharmacokinetics in the mentioned types of AMI, while STEMI patients may be at risk of having lower ticagrelor plasma concentration in the most crucial time during the early hours of AMI treatment.[18, 22] Similarly, potential differences in ticagrelor antiplatelet action between STEMI and NSTEMI have not been defined yet. Therefore, we decided to explore whether the pharmacokinetics and pharmacodynamics of ticagrelor differ between STEMI and NSTEMI patients. The Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT) study is expected to provide a valuable insight into our knowledge regarding the modern treatment of AMI patients.

METHODS AND ANALYSIS

Study objectives

The PINPOINT study is designed to compare the pharmacokinetics and pharmacodynamics of ticagrelor and its active metabolite (AR-C124910XX) in patients with STEMI and NSTEMI assigned to the invasive treatment.

Study design

The PINPOINT study is a phase IV, single centre, investigator-initiated, prospective, observational, pharmacokinetic/pharmacodynamic study. After admission to the study centre (Cardiology Clinic, Dr. A. Jurasz University Hospital, Bydgoszcz, Poland) and confirmation of STEMI or NSTEMI diagnosis according to the Third Universal Definition of Myocardial Infarction,[1] patients will be screened for eligibility for the study. Before any study specific procedure, each patient will provide a written informed consent to participate in the trial. All included patients will immediately receive orally a 300 mg LD of plain aspirin in integral tablets and a 180 mg LD of ticagrelor in integral tablets with 250 mL of tap water. Subsequently, all patients will promptly undergo coronary angiography followed by PCI, if required. Blood samples for pharmacokinetic and pharmacodynamic assessment will be drawn at eight predefined time points according to the blood sampling schedule already used at our site in a previous study (pre-treatment baseline, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - as shown in Figure 1).[31]

All enrolled patients with finally unconfirmed initial diagnosis of AMI will be excluded from the primary analysis. Patients qualified for urgent CABG within the blood sampling period also will not be included in the analysis. All study participants not receiving PCI will be reported.

Study population

The study population will include consecutive adult, male or non-pregnant female, P2Y12 receptor inhibitor-naive STEMI and NSTEMI patients, assigned to the invasive strategy. Full list of inclusion and exclusion criteria is presented in Table 1.

Table 1. Inclusion and exclusion criteria of the PINPOINT study.

Inclusion criteria	Exclusion criteria
• provision of informed consent prior to any study specific procedure	• treatment with ticlopidine, clopidogrel, prasugrel or ticagrelor within 14 days before the study enrolment
• diagnosis of STEMI or NSTEMI	• hypersensitivity to ticagrelor
• male or non-pregnant female, 18 years old and older	• current treatment with oral anticoagulant or chronic therapy with low-molecular-weight heparin
• provision of informed consent for angiography and PCI	• active bleeding
	• history of intracranial haemorrhage
	• fibrinolytic treatment during the index event
	• recent gastrointestinal bleeding (within 30 days)
	• history of coagulation disorders
	• history of moderate or severe hepatic impairment
	• history of major surgery or severe trauma (within 3 months)
	• second or third degree atrioventricular block during screening for eligibility
	• patient requiring dialysis
	• manifest infection or inflammatory state
	• Killip class III or IV during screening for eligibility
	• respiratory failure
	• current therapy with strong CYP3A inhibitors or strong CYP3A inducers

NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction.

Blood sample processing

Blood samples for the pharmacokinetic and pharmacodynamic evaluation will be obtained using a venous catheter (18G) inserted into a forearm vein at eight prespecified time-points (before ticagrelor LD, 30min, 1h, 2h, 3h, 4h, 6h and 12h post ticagrelor LD - Figure 1).

Venous blood for the pharmacokinetic evaluation will be collected into lithium-heparin vacuum test-tubes. Immediately after collection each sample will be placed on dry ice and transferred to the central laboratory. Subsequently, within 20 minutes from collection, blood specimens will be centrifuged at 1500 g for 12 minutes at 4ºC. Within 10 minutes post-centrifugation, obtained plasma samples will be stored at temperature below -60ºC until analyzed.

Venous blood for the assessment of pharmacodynamics with the VASP assay and multiple electrode aggregometry (MEA) will be collected into trisodium citrate and hirudin vacuum test-tubes, respectively. The first 3-5 mL of blood will be discarded to avoid spontaneous platelet activation. The pharmacodynamic analysis will be performed for each sample within 24h and 60min from blood collection for VASP and MEA, respectively.

Assessment of pharmacokinetics

Plasma concentration of ticagrelor and AR-C124910XX in samples obtained at all eight predefined time points (Figure 1) will be evaluated using liquid chromatography mass spectrometry coupled with tandem mass spectrometry, as previously described.[18, 32] Briefly, ticagrelor and AR-C124910XX will be extracted using 4ºC methanol solution containing [2H7]ticagrelor internal standard (TM-ALS-13-226-P1, ALSACHIM, France), while calibration curves will be obtained using ticagrelor (SVI-ALS-13-146, ALSACHIM, France) and AR-C124910XX (TM-ALS-13-193-P1, ALSACHIM, France) standards. Analysis will be performed using the Shimadzu UPLC Nexera X2 system consisting of LC-30AD pumps, SIL-30AC Autosampler, CTO-20AC column oven, FCV-20-AH2 valve unit, and DGU-20A5R degasser coupled with Shimadzu 8030 ESI-QqQ mass spectrometer. Lower limits of quantification are 4.69 ng/mL for both ticagrelor and AR-C124910XX.

Assessment of pharmacodynamics

Platelet VASP assay (Biocytex, Inc., Marseille, France) will be applied to all study participants at all predefined time points. MEA (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) will be used at all predefined time points (Figure 1) for all study participants with the exception of those treated with glycoprotein IIb/IIIa (GP IIb/IIIa) receptor inhibitors as this therapy may affect the results of platelet reactivity assessment performed with MEA (Figure 2). Pharmacodynamic assessment with VASP and MEA will be performed according to the manufacturers' instructions, as previously described.[33, 34] HPR will be defined as platelet reactivity index (PRI) >50% and area under the aggregation curve >46 units, when evaluated with VASP and MEA, respectively.[35]

Treatment

All patients included in the trial will be treated according to the current European Society of Cardiology (ESC) guidelines.[2, 3, 36] Standard therapy will include aspirin, ticagrelor, beta-blockers, statins, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, if not contraindicated. Morphine will be used at the discretion of the ambulance staff and the attending physician. The type of implanted stent and choice of the access site for coronary invasive procedure (radial or femoral) will be at the discretion of the operator. During the periprocedural period, all study participants will receive unfractionated heparin in body weight adjusted dose according to the ESC recommendations.[2, 3, 36] Administration of GP IIb/IIIa receptor inhibitors will be restricted only to bailout situations. Interventional cardiologists will be encouraged to use manual thrombectomy in case of visible thrombus.

Study endpoints

The primary endpoint of the study is area under the plasma concentration-time curve ($AUC_{(0–6)}$) for ticagrelor during the first 6 hours after the LD of ticagrelor. Secondary endpoints include $AUC_{(0–6)}$ for AR-C124910XX, area under the plasma concentration-time curve ($AUC_{(0–12)}$) for ticagrelor during the first 12 hours after the LD of ticagrelor, $AUC_{(0–12)}$ for AR-C124910XX, maximum concentration (C_{max}) of ticagrelor and AR-C124910XX, time to maximum concentration (t_{max}) for ticagrelor and AR-C124910XX, PRI assessed by the VASP assay, platelet reactivity assessed by MEA, percentage of patients with HPR after ticagrelor LD assessed with the VASP assay and MEA, time to reach platelet reactivity below the cut-off value for HPR evaluated with the VASP assay and MEA.

Statistical analysis

The continuous variables in both study groups will be compared using the t-test for normally distributed values as assessed by Kolmogorov-Smirnov test. Otherwise, the Mann-Whitney U test will be used. Proportions will be compared using the chi-square test when appropriate. A single linear regression analysis will be performed and will be followed by a multiple regression analysis if any variables are found to significantly affect the study primary endpoint. Pharmacokinetic calculations and plots will be made using dedicated software.

Determination of sample size

Since there is no reference study comparing the pharmacokinetics of ticagrelor in STEMI and NSTEMI patients, we decided to perform an internal pilot study of at least 15 patients with each type of AMI for estimating the final sample size. Eventually, the pilot study population comprised of 45 patients (15 with NSTEMI and 30 with STEMI). It included all participants consecutively entering the trial until the number of patients in the smaller group (NSTEMI) reached the prespecified minimal threshold.

The means and standard deviations of $AUC_{(0–6)}$ for ticagrelor in the first 30 STEMI patients and 15 NSTEMI patients were 2382 ± 2282 and 6406 ± 4082 ng*h/ml, respectively. Based on these results and assuming a two-sided alpha value of 0.05, we calculated, using the t-test for independent variables, that enrolment of at least 23 patients in each study arm would provide a 95% power to demonstrate a significant difference in $AUC_{(0–6)}$ for ticagrelor between patients with different types of MI.

Study limitations

Several limitations of our study have to be acknowledged. First, it is likely that the anticipated trial population will not be sufficient to evaluate clinical endpoints or perform subgroup analyses. Second, patients receiving morphine are not excluded from the study, which may result in differences in the baseline characteristics between the examined groups. Third, morphine is used at the discretion of the paramedics or the attending physicians, although we encourage the medical staff to administer a standardized dose of 5 mg intravenously, if required in any potential or actual study participant. On the other hand, even though it may be perceived as a limitation, this will enable us to obtain data in a real-world setting and will not create an artificially selected population.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the principles contained in the Declaration of Helsinki and Good Clinical Practice guidelines. The study received a favourable ethical opinion and approval from the Local Ethics Committee (Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy; study approval reference number KB 617/2015). Each patient will provide a written informed consent for participation in the study.

Safety

The following safety endpoints will be recorded during the blood sampling period: all-cause death, recurrent myocardial infarction according to the Third Universal Definition of Myocardial Infarction, stroke, and transient ischaemic attack according to definitions used in the PLATO trial, definite or probable stent thrombosis according to the Academic Research Consortium criteria, minor and major bleedings according to the Thrombolysis In Myocardial Infarction (TIMI) criteria, dyspnea adverse events according to criteria used in the PLATO trial, bradyarrhythmic events according to criteria used in the PLATO trial.

Present status

The approval of the Local Ethics Committee was obtained on September 29, 2015. On November 9, 2015 the PINPOINT study was registered on ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT02602444). The first patient was enrolled in November 2015. The baseline characteristics of patients included in the pilot study are presented in Table 2.

Table 2. Baseline characteristics of patients included in the internal pilot study.

	STEMI (n=30)	NSTEMI (n=15)	p value
Age [years]	62.3 ± 8.8	63.9 ± 9.7	0.51
Age ≥70 years	6 (20.0%)	4 (26.7%)	0.89
Female	6 (20.0%)	5 (33.3%)	0.53
BMI [kg/m2]	28.6 ± 4.1	27.8 ± 4.2	0.76
Hypertension	10 (33.3%)	10 (66.7%)	0.036
Diabetes mellitus	6 (20.0%)	2 (13.3%)	0.89
Dyslipidaemia	27 (90.0%)	14 (93.3%)	0.85
Current smoker	13 (43.3%)	5 (33.3%)	0.52
Prior MI	0	2 (13.3%)	n/a
Prior PCI	2 (6.7%)	3 (20.0%)	0.4
Prior CABG	0	0	n/a
Congestive heart failure	0	0	n/a

Nonhaemorrhagic stroke	0	0	n/a
Peripheral arterial disease	1 (3.3%)	2 (13.3%)	0.21
Chronic renal disease	0	0	n/a
Chronic obstructive pulmonary disease	0	0	n/a
Gout	1 (3.3%)	1 (6.7%)	n/a
Morphine use during current MI	17 (56.7%)	6 (40.0%)	0.29

BMI: body mass index; CABG: coronary artery bypass surgery; MI: myocardial infarction; n/a: not available; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction. Data are presented as mean ± standard deviation or number (%).

Dissemination of results

Results of the PINPOINT study will be disseminated through conference presentations and peer-reviewed journals. The results will also be available through the study record website at ClinicalTrials.gov.

SUMMARY

It is unknown whether ticagrelor pharmacokinetic profile and its antiplatelet effect are uniform in STEMI and NSTEMI patients, who are regarded in number of aspects as two distinct populations. The PINPOINT trial is expected to be the first study to elucidate whether STEMI is associated with poorer absorption and subsequently weaker antiplatelet action of ticagrelor in comparison with NSTEMI.

Contributors

JK and PA conceived the study. JK and PA wrote the study protocol with consultation from MO, JS, KO, KB, MKr, GS, MM and MKo. Subsequently JK, PA, MO, JS, KO, KB, MKr, GS, MM and MKo revised the manuscript critically for important intellectual content.

All the authors read and approved the final manuscript.

Competing interests

Dr. Jacek Kubica received a consulting fee from AstraZeneca. Dr. Marek Koziński received honoraria for lectures from AstraZeneca. All other authors have reported no relationships relevant to the contents of this paper.

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Ethics approval

Local Ethics Committee: Komisja Bioetyczna Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy (study approval reference number: KB 617/2015).

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Figure 1. The PINPOINT study schema.

ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

Figure 2. Platelet reactivity evaluation schedule for the PINPOINT study.

GP IIb/IIIa: glycoprotein IIb/IIIa; MEA: multiple electrode aggregometry; VASP: vasodilator-stimulated phosphoprotein.

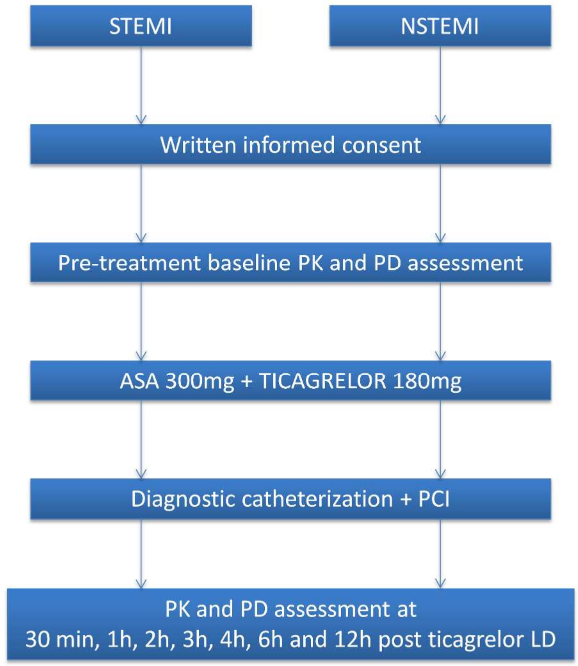

Figure 1. PINPOINT study schema.
ASA: aspirin; LD: loading dose; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; PD: pharmacodynamics; PK: pharmacokinetics; STEMI: ST-elevation myocardial infarction.

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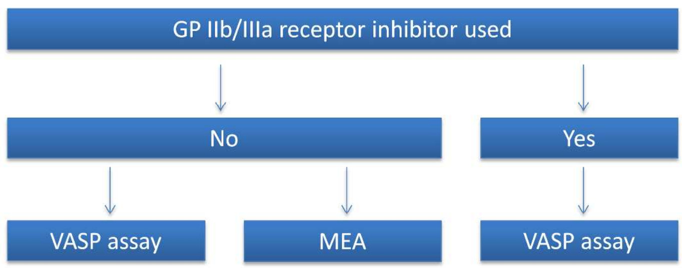

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GP IIb/IIIa: glycoprotein IIb/IIIa; MEA: multiple electrode aggregometry; VASP: vasodilator-stimulated phosphoprotein.

254x190mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

Protocol for the PINPOINT study.

		Item No	Recommendation
✓	**Title and abstract**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
	Introduction		
✓	Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
✓	Objectives	3	State specific objectives, including any prespecified hypotheses
	Methods		
✓	Study design	4	Present key elements of study design early in the paper
✓	Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✓	Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
✓	Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✓	Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias		9	Describe any efforts to address potential sources of bias
✓	Study size	10	Explain how the study size was arrived at
Quantitative variables		11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✓	Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Comment [PA1]: Will be published in the main publication with final results of the study.

Comment [PA2]: Will be published in the main publication with final results of the study.

Results			
✓ Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
✓ Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
✓ Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information			
✓ Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

Comment [PA3]: Will be published in the main publication with final results of the study.

Comment [PA4]: Will be published in the main publication with final results of the study.

Comment [PA5]: Will be published in the main publication with final results of the study.

Comment [PA6]: Will be published in the main publication with final results of the study.

Comment [PA7]: Will be published in the main publication with final results of the study.

Comment [PA8]: Will be published in the main publication with final results of the study.