PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): protocol for a prospective, observational, single centre study
AUTHORS	Adamski, Piotr; Ostrowska, Małgorzata; Sikora, Joanna; Obońska, Karolina; Buszko, Katarzyna; Krintus, Magdalena; Sypniewska, Grazyna; Marszałł, Michał; Kozinski, Marek; Kubica, Jacek

VERSION 1 - REVIEW

REVIEWER	Benjamin Hibbert University of Ottawa Heart Institute, Canada
REVIEW RETURNED	12-Sep-2016

GENERAL COMMENTS	I read with interest the study entitled “Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): a protocol for prospective, observational, single centre study”. This manuscript describes the protocol for a prospective single center study examining the pharmacological parameters of ticagrelor in patients with NSTEMI and STEMI. Ultimately, the topic undertaken is certainly interesting and timely given rapid advances in this field. However, this study is rather simple in terms of overall design and limited complexity. Hence, the value of a protocol paper for such a simple trial design is likely quite limited. Regardless, the following points could be considered: 1) Introduction a. The introduction is too long and could be condensed. b. A Brief review of ticagrelors metabolites and factors which impact antiplatelet effect c. The study’s acronym “PINPOINT” does not appear to be defined anywhere in the text. 2) Methods a. Will a standardized definition of STEMI/NSTEMI be employed for the purposes of the study or will it be at the clinician’s discretion? b. For the aspirin administration, will this be chewed or swallowed whole? c. How will patients taking P2Y12 agents prior to presentation with ACS be managed? d. The standardized adjuvant medical therapy is discussed; however, other medications known to influence antiplatelet metabolism are not discussed. Will opioids, proton pump inhibitors or H2 receptor blockers be excluded or adjusted for? e. What anticoagulation will be employed for angiography/PCI and with what protocols post-PCI? This should be clearly outlined. f. Does this center function solely as a primary PCI center or are patients transferred there as part of a pharmacoinvasive strategy as well? If so, one would assume patient’s receiving fibrinolytics would

	be excluded. This should be specified either way. g. HPLC-MS will be utilized for analysis of pharmacokinetics. Is this assay already developed? If so, reference to the previous work describing the fundamental methodology and sample processing would be of benefit. What is the fidelity of this assay? 3) Ethics and dissemination a. Safety endpoints. What duration of follow-up will be employed for safety endpoints? b. Only definite (not probable, possible) stent thrombosis as per ARC criteria are defined as safety endpoints. It would be of benefit to include all three classes to improve the granularity of the safety data collected. c. In Table 1, comparisons of each baseline characteristic should be provided and p-values noted in an adjacent column. d. Note is made in the preliminary data that there is a greater proportion of patients with prior MI and PCI in the NSTEMI arm. Do the author's have any insight into why this may be. Is there any knowledge on the time period since their prior PCI and pre-existing P2Y12 use? 4) Minor a. In general, the manuscript is reasonably well written. However, it would benefit from a thorough editorial review for English grammar, spelling and sentence structure. b. There is no discussion of anticipated limitations, challenges or shortcomings – this would be of benefit.

REVIEWER	Francesco Franchi University of Florida College of Medicine-Jacksonville. USA
REVIEW RETURNED	03-Oct-2016

GENERAL COMMENTS	The present manuscript by Adamski et al reports the study design and rationale of the PINPOINT study, which is aimed to investigate in a prospective fashion the difference in the PK and PD profiles of ticagrelor in STEMI vs. NSTEMI patients. The study deals with a topic of interest and the methods seem adequate to reach the study aim. However, I have some comments on the present study protocol manuscript that need to be addressed. Comments: 1. The introduction should be renamed into background and rationale. In this section the authors should focus more on the rationale of a different PK and PD of ticagrelor in STEMI vs NSTEMI, describing more in details possible reasons for this, as well as the studies showing a different PK/PD profile of ticagrelor in STEMI. Morphine administration is only one of the possible mechanisms and its role has not been clearly defined. The authors should reference the study by Franchi et al (JACC Cardiovasc Interv. 2015) showing that Tmax is long in STEMI patients, and that this occur with or without morphine. Instead, the first part of the introduction which broadly describe AMI epidemiology and management can be shortened. 2. The authors should specify the choice of parenteral anticoagulant during PCI. 3. The authors should specify how they will consider patients enrolled for STEMI and pretreated with ticagrelor but not receiving PCI (e.g. false activation, different diagnosis, CABG). Will these patients be excluded from the analysis? 4. The authors should specify if they plan to perform a multivariable analysis, or if they have any other statistical plan to account for the

	differences in baseline characteristics that there will be between STEMI and NSTEMI patients. The differences in this populations are well-known and may affect PK/PD profiles of ticagrelor. 5. The authors should specify how HPR will be defined. 6. The authors should provide more specific and detailed methods about PK analysis and PD assays.

REVIEWER	Manne Holm Karolinska institutet, Sweden I have together with my co-author Jan van der Linden previously received a non-restricted research grant from AstraZeneca. Moreover I have previously received an honorarium from Roche Diagnostics for a lecture.
REVIEW RETURNED	05-Oct-2016

GENERAL COMMENTS	Adamski P et al has submitted a protocol for an ongoing study on the difference in ticagrelor pharmacokinetics and pharmacodynamics between patients presenting with STEMI and NSTEMI. Overall the study is interesting and the protocol easy to follow. I have some suggestions that may improve the manuscript: Major: 1. In the last few years, STEMI patients have been found to have a delayed onset of platetel inhibition not only with clopidogrel, but also with ticagrelor and prasugrel. Impaired GI motility due to morphine treatment has been proposed as a major factor for this delayed onset of effect. This has indeed been showed both in observational studies (e.g. RAPID, PRIVATE-ATLANTIC) and in randomized studies (e.g. IMPRESSION). Studies conducted in Sweden during 2014 -2016 (not yet published data) has reported a morphine treatment rate of around 90% in patients presenting with STEMI compared with around 20% in patients presenting with NSTEMI. In my opinion, the authors should have a strategy on how to address this factor as it most probably is much more common among STEMI patients. Otherwise they won't be able to tell if a delay in the ticagrelor pharmacokinetics/dynamics was caused by the condition of STEMI or morphine treatment. I would suggest adressing this with either a stratification or the addition of an exclusion criterion. Moreover, any morphine treatment in the already included patients should be presented in table 2. 2. The criteria for NSTEMI must be better specified. Do the authors include patients with symptoms and ST-depression on the ECG before troponin levels are measured? Do they wait for heart biomarkers? This must be addressed in order to understand how the inclusion is performed as they otherwise should address the NSTEMI group as NSTE-ACS, which includes troponin negative patients with unstable angina. 3. When handling blood samples for evaluation of drug concentrations, it is very important to properly cool them before spinning them in a refrigerated centrifuge. Moreover, the time to centrifugation must not be too long and especially if no pre-cooling on ice or in a refrigerator is done. Do the authors handle samples after each sampling time or pool them together and centrifuge all the

	sample after the 12 hour sampling? Please expand on how this was performed as it is not described in enough detail in the current version of the protocol. This is also true for the pharmacodynamic sampling (e.g. MEA must be performed within 2 hours for trustworthy results). Minor: 1. The introduction may easily be shortened removing the first five or six paragraphs. 2. The blood sampling time points should be provided in the abstract. 3. The tables may be esthetically improved.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Benjamin Hibbert
Institution and Country: University of Ottawa Heart Institute, Canada
Competing Interests: None Declared
I read with interest the study entitled “Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): a protocol for prospective, observational, single centre study”. This manuscript describes the protocol for a prospective single center study examining the pharmacological parameters of ticagrelor in patients with NSTEMI and STEMI. Ultimately, the topic undertaken is certainly interesting and timely given rapid advances in this field. However, this study is rather simple in terms of overall design and limited complexity. Hence, the value of a protocol paper for such a simple trial design is likely quite limited. Regardless, the following points could be considered:

1) Introduction
Q: a. The introduction is too long and could be condensed.

A: We would like to thank for this comment. We did our best to modify the introduction to include all suggestions and instructions from all three reviewers. Below please find the corrected introduction:

'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.'

Q: b. A Brief review of ticagrelors metabolites and factors which impact antiplatelet effect

A: The requested information was added and can be found in 'Background' and 'Rationale' sections of the introduction. Please see answer to request 1/a.

Q: c. The study’s acronym “PINPOINT” does not appear to be defined anywhere in the text.

A: We would like to thank the reviewer for this observant remark. The study's acronym has now been defined in the text - the Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT) study.

2) Methods
Q: a. Will a standardized definition of STEMI/NSTEMI be employed for the purposes of the study or will it be at the clinician’s discretion?

A: To clarify this issue we modified the 'Study design' paragraph to include: '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.'

Q: b. For the aspirin administration, will this be chewed or swallowed whole?

A: Aspirin will be swallowed whole. To elucidate this matter the 'Study design' section has been modified as follows: 'All included patients will immediately receive orally a 300 mg LD of plain aspirin in integral tablets ... '

Q: c. How will patients taking P2Y12 agents prior to presentation with ACS be managed?

A: Use of any P2Y12 receptor antagonist within 2 weeks prior the index event is the primary exclusion criterion, which is stated in Table 1 - Inclusion and exclusion criteria of the PINPOINT study (Exclusion criteria: 'treatment with ticlopidine, clopidogrel, prasugrel or ticagrelor within 14 days before the study enrolment'). To underline this fact 'Study population' section has been modified as follows:

'The study population will include consecutive adult, male or non-pregnant female, P2Y12 receptor inhibitor-naive STEMI and NSTEMI patients, designated to invasive strategy.'

Q: d. The standardized adjuvant medical therapy is discussed; however, other medications known to influence antiplatelet metabolism are not discussed. Will opioids, proton pump inhibitors or H2 receptor blockers be excluded or adjusted for?

A: We would like our trial to resemble 'real life patients' as much as possible, therefore opioid use during the index event will not exclude patients from the study ('Morphine will be used at the discretion of the ambulance staff and the attending physician.' was added to 'Treatment' section to underline it). We expect more STEMI than NSTEMI patients to receive morphine and in case the difference in opioid administration between the study groups is statistically significant, the results will be adjusted for. However, as we are enrolling NSTEMI patients designated to invasive strategy, who in our centre are mainly very high and high risk patients, and who often require analgesia due to ongoing chest pain, we expect to observe reasonably frequent morphine use in this group too. With regard to anti-acid medications, it is true that according to a post-hoc analysis of the PLATO study, the use of PPIs or H2 receptor antagonists in the ticagrelor arm was associated with increased risk of cardiovascular events. However, our trial is a small, purely PK/PD study with few hours follow-up, therefore in the light of no studies reporting interaction between PPIs or H2 receptor blockers and ticagrelor PK/PD, we do not consider this issue to have potential to influence the results. Nevertheless, the use of PPIs and H2 receptor blockers will be recorded and compared between the examined groups to verify that.

Q: e. What anticoagulation will be employed for angiography/PCI and with what protocols post-PCI? This should be clearly outlined.

A: We would like to thank for this important comment. The following information has been added to 'Treatment' section: 'During the periprocedural period, all study participants will receive unfractionated heparin in body weight adjusted dose according to the ESC recommendations.'

Q: f. Does this center function solely as a primary PCI center or are patients transferred there as part of a pharmacoinvasive strategy as well? If so, one would assume patients receiving fibrinolytics would be excluded. This should be specified either way.

A: Our site is a high volume primary PCI center. With a well developed cath lab network in Poland, at present we generally do not encounter AMI patients treated with fibrinolysis. Such patients would not be considered as potential trial participants, thus to address the raised issue we have added 'fibrinolytic treatment during the index event' to the study exclusion criteria listed in Table 1.

Q: g. HPLC-MS will be utilized for analysis of pharmacokinetics. Is this assay already developed? If so, reference to the previous work describing the fundamental methodology and sample processing would be of benefit. What is the fidelity of this assay?

A: As requested we cited papers describing the methodology of ticagrelor and AR-C124910XX PK determination used at our center, elaborated on sample processing and provided assay fidelity: '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. (...) 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.'

3) Ethics and dissemination
Q: a. Safety endpoints. What duration of follow-up will be employed for safety endpoints?

A: Safety endpoints will be recorded during the blood sampling period (the first 12 hours after ticagrelor loading dose). We modified 'Safety' section to include the information on the duration of follow-up: 'The following safety endpoints will be recorded during the blood sampling period ... '

Q: b. Only definite (not probable, possible) stent thrombosis as per ARC criteria are defined as safety endpoints. It would be of benefit to include all three classes to improve the granularity of the safety data collected.

A: As suggested we have added probable stent thrombosis to the safety points that will be recorded and reported. Due to 12 hour duration of the follow-up it is not possible to record possible stent thrombosis, which according to its definition should be considered in case of death >30 days post-PCI.

Q: c. In Table 1, comparisons of each baseline characteristic should be provided and p-values noted in an adjacent column.

A: P values were omitted intentionally as the table presents only preliminary data. Nevertheless, as requested we have provided a p value for each baseline characteristic.

Q: d. Note is made in the preliminary data that there is a greater proportion of patients with prior MI and PCI in the NSTEMI arm. Do the author’s have any insight into why this may be. Is there any knowledge on the time period since their prior PCI and pre-existing P2Y12 use?

A: The reported pilot study baseline characteristics correspond well with our everyday experience where STEMI patients tend to be younger and generally 'healthier', whereas NSTEMI patients usually are older and have more co-morbidities, including risk factors for ischaemic heart disease (hypertension, diabetes, etc.), which attribute to higher prevalence of previous MI and/or PCI in this group. However, as our study is not powered to draw any epidemiological conclusions, we would prefer not to comment on this issue, especially that the differences mentioned by the reviewer are not statistically significant between pilot study participants. Regarding the time period since prior PCI - none of the previous PCIs was performed within 12 months before the inclusion to the current trial. Use of any P2Y12 receptor antagonists within 14 days before the current MI is the main exclusion criterion for the study (Table 1), therefore all study participants are P2Y12 blocker-naive at the moment of enrolment. To underline this fact 'Study population' section has been modified as follows: 'The study population will include consecutive adult, male or non-pregnant female, P2Y12 receptor inhibitor-naive STEMI and NSTEMI patients, designated to invasive strategy.'

4) Minor

Q: a. In general, the manuscript is reasonably well written. However, it would benefit from a thorough editorial review for English grammar, spelling and sentence structure.

A: As suggested the manuscript underwent an additional review to improve the quality of English used in the text.

Q: b. There is no discussion of anticipated limitations, challenges or shortcomings – this would be of benefit.

A: We would like to thank the reviewer for this remark. We initially planned to include this section in the manuscript reporting the final results of our study, but we agree that inclusion of 'Study limitations' section will improve the study protocol. Therefore, the following has been added to the manuscript: '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 discretion of paramedics or 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.'

Reviewer: 2
Reviewer Name: Francesco Franchi
Institution and Country: University of Florida College of Medicine-Jacksonville. USA.
Competing Interests: None declared
The present manuscript by Adamski et al reports the study design and rationale of the PINPOINT study, which is aimed to investigate in a prospective fashion the difference in the PK and PD profiles of ticagrelor in STEMI vs. NSTEMI patients. The study deals with a topic of interest and the methods seem adequate to reach the study aim. However, I have some comments on the present study protocol manuscript that need to be addressed.

Comments:
Q: 1. The introduction should be renamed into background and rationale. In this section the authors should focus more on the rationale of a different PK and PD of ticagrelor in STEMI vs NSTEMI, describing more in details possible reasons for this, as well as the studies showing a different PK/PD profile of ticagrelor in STEMI. Morphine administration is only one of the possible mechanisms and its role has not been clearly defined. The authors should reference the study by Franchi et al (JACC Cardiovasc Interv. 2015) showing that Tmax is long in STEMI patients, and that this occur with or without morphine. Instead, the first part of the introduction which broadly describe AMI epidemiology and management can be shortened.

A: 'Introduction' section is requested per journal style, but we divided it into two subsections (background and rationale) as suggested. We did our best to compliy to all the above comments and instructions. To see the updated 'Introduction' with all the requested corrections please refer to our answer to query 1/a by the reviewer 1.

Q: 2. The authors should specify the choice of parenteral anticoagulant during PCI.

A: We would like to thank for this observant and important comment. The following information has been added to 'Treatment' section: 'During the periprocedural period, all study participants will receive unfractionated heparin in body weight adjusted dose according to the ESC recommendations.'

Q: 3. The authors should specify how they will consider patients enrolled for STEMI and pretreated with ticagrelor but not receiving PCI (e.g. false activation, different diagnosis, CABG). Will these patients be excluded from the analysis?

A: Patients with not confirmed MI diagnosis will be excluded from the final analysis. Patients who will require CABG within the blood sampling period also will not be included in the analysis - for technical reasons, we are not able to continue blood collection after the patient is transferred to the cardiac surgery unit. We added the following to 'Study design' section to address the raised issues: '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.'

Q: 4. The authors should specify if they plan to perform a multivariable analysis, or if they have any other statistical plan to account for the differences in baseline characteristics that there will be between STEMI and NSTEMI patients. The differences in this populations are well-known and may affect PK/PD profiles of ticagrelor.

A: We would like to thank the reviewer for this comment. As suggested we specified our statistical approach. '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.' was added to the study protocol.

Q: 5. The authors should specify how HPR will be defined.

A: We are very grateful for this remark. The definitions of HPR for VASP and MEA have been added to the manuscript: '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]'

Q: 6. The authors should provide more specific and detailed methods about PK analysis and PD assays.

A: As requested we provided more detailed description on PK and PD analysis methods:
'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]'

Reviewer: 3
Reviewer Name: Manne Holm
Institution and Country: Karolinska institutet, Sweden
Competing Interests: I have together with my co-author Jan van der Linden previously received a non-restricted research grant from AstraZeneca. Moreover I have previously received an honorarium from Roche Diagnostics for a lecture.
Adamski P et al has submitted a protocol for an ongoing study on the difference in ticagrelor pharmacokinetics and pharmacodynamics between patients presenting with STEMI and NSTEMI. Overall the study is interesting and the protocol easy to follow. I have some suggestions that may improve the manuscript:

Major:
Q: 1. In the last few years, STEMI patients have been found to have a delayed onset of platetel inhibition not only with clopidogrel, but also with ticagrelor and prasugrel. Impaired GI motility due to morphine treatment has been proposed as a major factor for this delayed onset of effect. This has indeed been showed both in observational studies (e.g. RAPID, PRIVATE-ATLANTIC) and in randomized studies (e.g. IMPRESSION). Studies conducted in Sweden during 2014 -2016 (not yet published data) has reported a morphine treatment rate of around 90% in patients presenting with STEMI compared with around 20% in patients presenting with NSTEMI. In my opinion, the authors should have a strategy on how to address this factor as it most probably is much more common among STEMI patients. Otherwise they won't be able to tell if a delay in the ticagrelor pharmacokinetics/dynamics was caused by the condition of STEMI or morphine treatment. I would suggest adressing this with either a stratification or the addition of an exclusion criterion. Moreover, any morphine treatment in the already included patients should be presented in table 2.

A: We would like to thank the reviewer for this comment. We considered this relevant issue when initially planning this trial. Eventually, we wanted our study to resemble 'real life patients' as much as possible, therefore opioid administration during the index event will not exclude patients from the study ('Morphine will be used at the discretion of the ambulance staff and the attending physician.' was added to Treatment section to underline it). We obviously expect more STEMI than NSTEMI patients to receive morphine, which was addressed in newly added 'Study limitations' ('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.'). In case the difference in opioid administration between the study groups is statistically significant, the study results will be adjusted for and a single/multiple regression analysis will be performed, which was added to Statistical analysis section (please also see our answers to comments 2/d by reviewer 1 and 4 by reviewer 2). As requested we also presented the prevalence of morphine administration in already included patients (Table 2).

Q: 2. The criteria for NSTEMI must be better specified. Do the authors include patients with symptoms and ST-depression on the ECG before troponin levels are measured? Do they wait for heart biomarkers? This must be addressed in order to understand how the inclusion is performed as they otherwise should address the NSTEMI group as NSTE-ACS, which includes troponin negative patients with unstable angina.

A: The NSTEMI diagnosis among study participants is made according to the Third Universal Definition of Myocardial Infarction, which was added to the text for clarification. As the mentioned

document states, we make NSTEMI diagnosis if at least one cardiac troponin measurement is above the 99th percentile upper reference limit and at least one of the following is present: symptoms of ischaemia, new ST-T changes, development of pathological Q waves or evidence of new regional wall motion abnormalities in transthoracic echocardiography. Therefore, if a patient with NSTE-ACS requires coronary angiography before troponin concentration is available, such patient does not fulfil inclusion criteria. If the reviewer requires, this explicit description will be added to the manuscript, however as the Third Universal Definition of Myocardial Infarction is a commonly known document, we believe that citation of this document is sufficient. Of note, at our site all potential NSTE-ACS patients have troponin concentration returned by the lab within 60 minutes from presentation, so NSTE-ACS patients undergoing coronary angiography without troponins available occur very seldom.

Q: 3. When handling blood samples for evaluation of drug concentrations, it is very important to properly cool them before spinning them in a refrigerated centrifuge. Moreover, the time to centrifugation must not be too long and especially if no pre-cooling on ice or in a refrigerator is done. Do the authors handle samples after each sampling time or pool them together and centrifuge all the sample after the 12 hour sampling? Please expand on how this was performed as it is not described in enough detail in the current version of the protocol. This is also true for the pharmacodynamic sampling (e.g. MEA must be performed within 2 hours for trustworthy results).

A: We would like to thank for this remark and we agree we should have addressed this issue the primary version of the protocol. Therefore, we added a separate section on 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.'

Minor:
Q: 1. The introduction may easily be shortened removing the first five or six paragraphs.

A: We complied to the suggestions and instructions by all three reviewers . To see the updated 'Introduction' with all the requested corrections please refer to query 1/a by reviewer 1.

Q: 2. The blood sampling time points should be provided in the abstract.

A: We would like to thank the reviewer for this comment. Detailed sampling schedule has been added to the abstract as requested.

Q: 3. The tables may be esthetically improved.

A: We provided raw tables with intention that they will be edited and adjusted per journal style by the BMJ Open staff during the publication process. Nevertheless, as requested we tried to modify tables' outlay to improve them esthetically. We welcome any suggestions in case the reviewer feels further improvement is necessary.

VERSION 2 – REVIEW

REVIEWER	Benjamin Hibbert University of Ottawa Heart Institute
REVIEW RETURNED	07-Nov-2016

GENERAL COMMENTS	I reviewed the revisions for the study entitled “Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): a protocol for a prospective, observational, single centre study”. The author’s have made a good effort to address all of the points raised by the review process. As mentioned previously, the topic remains quite interesting and timely. However, this remains a small, single centre, observational study which is relatively simple in design. Hence, the incremental benefit of publishing a protocol paper just to describe the methodology of a straightforward study remains limited. The results of this study will likely be quite interesting nonetheless and when the final results are available, incorporation of the methods into the overall final paper would seem a more appropriate avenue for this information. As such, despite the author’s commendable efforts to address all of the points listed, I would still recommend not pursuing publication given this study adds little to the literature by itself. I look forward to the final results once the study is completed. Thank you for the opportunity to review this interesting work.

REVIEWER	Francesco Franchi University of Florida College of Medicine-Jacksonville, USA
REVIEW RETURNED	17-Nov-2016

GENERAL COMMENTS	The present manuscript by Adamski et al is a revised submission. The authors have fairly addressed reviewers' comments and the manuscript has significantly improved. I don't have any further comment at this time.

REVIEWER	Manne Holm Karolinska Institutet, Sweden I have together with my co-author Jan van der Linden previously received a non-restricted research grant from AstraZeneca. Moreover I have previously received an honorarium from Roche Diagnostics for a lecture.
REVIEW RETURNED	04-Nov-2016

GENERAL COMMENTS	To summarize, the author provided satisfactory answers to my previous questions and edited their manuscript thereafter. I do still think that the introduction may still be shortened substantially to only address the relevant pharmacokinetic studies on ticagrelor and the rationale/aim of the study. I agree with the authors regarding their reasoning on morphine threatment and obtaining real life data and that this has been added

	as a limitation. My suggestion regarding specification of the criteria for NSTEMI was not questioning the clinical practice used at the including clinic, but instead ment to clarify for other researchers reading this protocol if they would like to conduct a similar study. I am aware of clinics where a ticagrelor loading dose is administered in patients with "suspected NSTEMI" i.e. with relevant symptoms together with ST-T changes on the ECG (but before receiving the cardiac markers from the laboratory). If one would include such a patient before their ticagrelor LD and the cardiac markers prover negative (together with the serial blood sampling), the inclusion criteria NSTEMI would not be fulfilled.The authors did, however, answer their practice of administering a loading dose in a satisfactory way so that the NSTEMI patients in the study all have known pathological cardiac markers before inclusion.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Benjamin Hibbert
Institution and Country: University of Ottawa Heart Institute, Canada
Competing Interests: None declared

Q: I reviewed the revisions for the study entitled “Comparison of Ticagrelor Pharmacokinetics and Pharmacodynamics in STEMI and NSTEMI Patients (PINPOINT): a protocol for a prospective, observational, single centre study”. The author’s have made a good effort to address all of the points raised by the review process. As mentioned previously, the topic remains quite interesting and timely.

However, this remains a small, single centre, observational study which is relatively simple in design. Hence, the incremental benefit of publishing a protocol paper just to describe the methodology of a straightforward study remains limited. The results of this study will likely be quite interesting nonetheless and when the final results are available, incorporation of the methods into the overall final paper would seem a more appropriate avenue for this information.

As such, despite the author’s commendable efforts to address all of the points listed, I would still recommend not pursuing publication given this study adds little to the literature by itself.

I look forward to the final results once the study is completed. Thank you for the opportunity to review this interesting work.

A: We would like to thank Dr Hibbert for his thorough review of our study protocol and its revision. We greatly appreciate the acknowledgment we received for our efforts to improve the manuscript and to address all the raised points. Moreover, we are very content that the reviewer finds our research interesting and timely. However, in the face of positive evaluation of our work by all three reviewers, including Dr Hibbert, we are dejected to see that the first reviewer does not find justification for publication of the proposed study protocol. We are aware of several shortcomings of our trial (which we listed in 'Study limitations' section) and we value the reviewer's opinion, but in this place we would like to oppose. Undoubtedly our trial is much less numerous compared with large clinical trials, but it has to be underlined that it is purely pharmacokinetic/pharmacodynamic (PK/PD) study. The majority of PK/PD studies on antiplatelet agents published in top cardiology journals are of comparable size with the current study. The observational character of the described trial obviously derives from the researched topic (comparison of ticagrelor PK/PD properties in patients with two different types of myocardial infarction). The trial is prospective and can be characterized by numerous PK and PD end

points assessed with several methods, which require exact and detailed description. Finally, at our clinical centre we follow internal quality standards of conveying clinical trials, including full transparency of our research, which we believe can only be accomplished by prospective trial registration together with publication of study protocols in open access journals.

Reviewer: 2
Reviewer Name: Francesco Franchi
Institution and Country: University of Florida College of Medicine-Jacksonville, USA
Competing Interests: None declared

Q: The present manuscript by Adamski et al is a revised submission. The authors have fairly addressed reviewers' comments and the manuscript has significantly improved. I don't have any further comment at this time.

A: We would like to thank Prof. Franchi for his positive review.

Reviewer: 3
Reviewer Name: Manne Holm
Institution and Country: Karolinska Institutet, Sweden
Competing Interests: I have together with my co-author Jan van der Linden previously received a non-restricted research grant from AstraZeneca. Moreover I have previously received an honorarium from Roche Diagnostics for a lecture.

Q: To summarize, the author provided satisfactory answers to my previous questions and edited their manuscript thereafter. I do still think that the introduction may still be shortened substantially to only address the relevant pharmacokinetic studies on ticagrelor and the rationale/aim of the study.

I agree with the authors regarding their reasoning on morphine treatment and obtaining real life data and that this has been added as a limitation.

My suggestion regarding specification of the criteria for NSTEMI was not questioning the clinical practice used at the including clinic, but instead ment to clarify for other researchers reading this protocol if they would like to conduct a similar study. I am aware of clinics where a ticagrelor loading dose is administered in patients with "suspected NSTEMI" i.e. with relevant symptoms together with ST-T changes on the ECG (but before receiving the cardiac markers from the laboratory). If one would include such a patient before their ticagrelor LD and the cardiac markers proved negative (together with the serial blood sampling), the inclusion criteria NSTEMI would not be fulfilled.The authors did, however, answer their practice of administering a loading dose in a satisfactory way so that the NSTEMI patients in the study all have known pathological cardiac markers before inclusion.

A: We greatly appreciate the positive evaluation of revised version of our manuscript by Dr Holm. As suggested we further shortened the introduction by 270 words to focus on the relevant pharmacokinetic studies on ticagrelor and the rationale for the current trial, but at the same time to include all the information required by the reviewers during the previous revision. Below please find modified version of the introduction.

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.

VERSION 3 – REVIEW

REVIEWER	Manne Holm Karolinska Institutet, Dept of Molecular Medicine and Surgery
REVIEW RETURNED	25-Jan-2017

GENERAL COMMENTS	The authors have performed adequate changes to the manuscript. No further comments.