The role of platelet tests in detection of clopidogrel hyper-response after percutaneous coronary intervention

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The role of platelet function tests in detection of Clopidogrel hyper-response after percutaneous coronary intervention

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Clopidogrel on Platelet function

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1. Summary

Clopidogrel is a PY212 antiplatelet medication which was shown to reduce mortality in patients post PCI. A percentage of the population has a resultant low platelet reactivity (LPR) or high platelet reactivity (HPR) while taking Clopidogrel. There is a connection between the LPR and increased rates of bleeding events as well as a connection between HPR and increased rates of ST events. We will examine bleeding avoidant strategies (BAS) which may be implemented to reduce bleeding event in those that are at high risk. The genetic polymorphism CYP2C19 and its association with LPR and HPR will also be looked at in more depth. Lastly, included in the discussion is the risk associated with LPR and bleeding events and HPR and ST events post PCI on clopidogrel and if these events can be accurately predicted with platelet reactivity testing before they occur. By exploring the different aspects of Clopidogrel and its resultant effects on LPR or HPR post PCI, different conclusions can be postulated on the best clinical strategies.

2. INTRODUCTION

Thrombocytes are an integral part of the human body whose main function is to arrest the bleeding from injured blood vessels, also known as hemostasis. There is a three step cascade which ultimately results in the closure of the injured site, adhesion, activation and aggregation. They do so by first identifying where the blood vessel is injured through the exposure of the endothelium. Attached to the endothelium is von Willebrand factor which allows the platelets to be anchored to the endothelium. The activation portion of the platelet cascade is under the influence of ADP and calcium efflux. An exposed endothelium releases prostacyclin which results in an efflux of calcium, promoting platelet activation. On the other side of platelet activation, a covered endothelium results in continuous release of endothelial-ADPase which inhibits ADP from activating the platelet cascade. In the occurrence of blood vessel injury ADP is freely able to bind to purinergic receptors PY212 resulting in further efflux of Calcium. The binding of ADP to the ADP receptor induces an expression of the GpIIb/IIIa receptor at the platelets surface. Fibrinogen binds to these GpIIb/IIIa receptors and allows the aggregation of these platelets and results in the arrest of the bleeding. (1) Although the function of platelets is to prevent bleeding from an injured blood vessel, platelet activation and aggregation play a role in occlusion of a blood vessel during a myocardial infarction and post percutaneous coronary intervention (PCI) stent thrombosis. Due to platelets involvement in these scenarios various drugs on the market act upon this platelet activation cascade to prevent the progression of blood vessel occlusion, one of which is Clopidogrel.

Clopidogrel is a platelet activation inhibitor which prevents the activation of the PY212 receptor by ADP. Due to Clopidogrels function in preventing platelet activation some of its medical applications include prevention of the occurrence of myocardial infarctions and stroke in high risk patients. The CURE trial has shown a significant decrease in mortality in patients using Clopidogrel post myocardial infarction. (2) It also plays a role in preventing stent thrombosis in patients that have undergone percutaneous coronary intervention (PCI). Clopidogrel is a drug that has been used for many years for pharmaceutical application and continues to be used around the world. The use of Clopidogrel post PCI is recommended within the European society of cardiology due to its proven benefit in cardiovascular related deaths. (3) Although a majority of the post-PCI patients that use dual antiplatelet therapy (DAT) following the guidelines have a positive reaction and ultimately decrease chance of mortality, there is growing evidence of higher bleeding complications. (31) This in turn leads to an increase in mortality in this subpopulation of patients which is similar to the mortality of patients with post PCI myocardial infarction. (32)

A number of genes have been identified that predispose patients to adverse reactions while taking Clopidogrel, CYP2C19*2, CYP2C19*17, ABCB1, most commonly being the CYP2C19 gene polymorphism. The hepatic oxidative process that activates Clopidogrel is a two step process that involve the highly polymorphic monooxygenase family, P450. It was uncovered that the CYP2C19 enzyme is associated with a gain of function or a loss of function variants. (4-6) All these genetic factors play either a role in the metabolism or absorption of Clopidogrel ultimately affecting the overall function of this drug on platelet function. Clopidogrel is a prodrug therefore it requires the enzyme CYP2C19 to oxidize Clopidogrel in

order to activate it. If there is an increase in function of this enzyme it will result in over activation of Clopidogrel and ultimately an increased risk of bleeding. If there is a decrease in enzyme function it has the opposite effect, decrease in Clopidogrel activation and an increase in platelet aggregation. Therefore, with these CYP2C19 genetic polymorphisms there can either be a gain of function or loss of function resulting in an enhanced or diminished platelet reactivity.

The ultimate goal of antiplatelet therapy is to have a platelet reactivity that is neither too high or too low. Too low of a platelet reactivity may lead to ST or Myocardial events. *It is the purpose of this paper to show those that are vulnerable to obtaining a high platelet reactivity (HPR) and low platelet reactivity (LPR) while placed on Clopidogrel. Another purpose of this paper is to view the risks involved in having both a HPR and LPR on Clopidogrel and to understand what platelet reactivity testing is required to reduce these risks.*

European society of cardiology released an article explaining the role of platelet function testing in those people that are undergoing PCI. The role of antiplatelet therapy in patients is to optimize their coagulation so that they don’t have either a low platelet function nor a high platelet function. It is difficult to obtain a platelet reactivity that is not too high nor too low due to the high variability in patient to patient reactivity to Clopidogrel. Although there is no determined methodology for interpreting the benefits of measuring platelet reactivity. *The aim of this study is also to determine an appropriate methodology, evaluation and interpretation of platelet reactivity.*

2.1 Patients with increased risk of bleeding Post PCI

There was a study that was conducted that tried to ascertain which patients would have an increased risk of bleeding events post PCI. This information can be useful in order to implement some bleeding avoidant strategies (BAS) before and after PCI. Some BAS include bivalirudin, radial approach, routine use of PPI (proton pump inhibitors) and vascular closure devices in the case of femoral approach. (27-29) Among these patients that have suffered a myocardial infarction, some of these BAS have shown a reduction in mortality. (30) In this study they included data from 1,043,759 PCI procedures in order to identify factors associated with bleeding events within 72hrs post PCI. Of these 1,043,759 patients there were 60,194 patients that had post PCI bleeding, meaning that the post PCI bleeding events were at 5.8%. It was shown that the most predictive factors of bleeding events were female sex followed by shock or salvage PCI and the least predictive were non insulin dependant diabetics. In this study they incorporated the NCDR CathPCI Bleeding Risk Score. This score used multiple variables such as age, sex, BMI to come up with a bleeding score out of 210. They used this data and compared it to all patients and viewed the correlation. They split these patients among three groups, high risk, medium risk and low risk. It was uncovered in the high risk group, the bleeding events were at 14%, at medium risk the bleeding events were at 4% and at low risk the bleeding events were below 2%. This score can provide a feedback for possible bleeding events involved in PCI relative to each patient's risk factors. It is with this score that one can determine which patients may require BAS and which do not. (34)

There was another study that was conducted that discussed the CRUSADE scoring. This scoring is based on 8 factors that pose a risk to bleeding events, in patients that have been treated for non ST elevated myocardial infarction (NSTEMI). The 8 factors that were used in the CRUSADE scoring are as follows, female sex, history of diabetes, prior vascular disease, heart rate, systolic blood pressure, signs of congestive heart failure, baseline hematocrit <36%, and creatinine clearance. Based on these 8 factors a scoring from 0 to 100 was performed on each individual patient and they were divided into 5 separate groups, very low risk which is a score from 0-20, low risk which is a score from 21-30, moderate risk which is a score from 31-40, high risk which is a score from 41-50 and very high risk which is 50 and above. Through this trial is was shown that those patients that fell into the very low risk category had a bleeding risk of 3.1%, those in the low risk group had a bleeding risk of 5.5%, those in the moderate risk group had a bleeding risk of 8.6%, those in the high risk group had a bleeding risk of 11.9% and those that were in the very high risk group had a bleeding risk of 19.5%. This data clearly shows an increase in bleeding events with increase risks factors that are present. It is with this study that one can debate the usage of BAS in patients with multiple risk factors that will undergo treatment for NSTEMI. (35)

2.2.CURRENT RECOMMENDATIONS

According to the guidelines by the ESC (European society for cardiology) in 2011 it is recommended that ticagrelor and prasugrel be used instead if Clopidogrel in NSTEMI (Non ST elevated myocardial infarction) and STEMI (ST elevated Myocardial Infarction) ACS (acute coronary syndrome). Although it is recommended to use ticagrelor and prasugrel, Clopidogrel is still classified as a class I P2Y12-inhibitor due to the high variability in availability of P2Y12-inhibitor within different European countries. Although the ACCF/AHA 2012/2013 guidelines don't explicitly approve of the use of ticagrlor and prasugrel opposed to Clopidogrel in NSTEMI AND STEMI due to the limited randomized clinical trials that have been conducted on ticagrelor and prasugrel. Also ACCF/AHA/SCAI guidelines have no preference of ticagrelor or prasugrel versus Clopidogrel in PCI (percutaneous coronary intervention). (7)

In regards to platelet reactivity the ECS placed a class IIb indication for platelet reactivity while on Clopidogrel because its use may be indicated in specific cases but on routine use it was shown that it results in no clinical benefit. ACCF/AHA/SCAI guidelines for PCI indicated that platelet reactivity testing may be done in very selective patients such as those that are high risk while on Clopidogrel. If HPR (high platelet reactivity) is shown, then the patient can be switched to either ticagrelor of prasugrel. (7-9)

2.3.PLATELET REACTIVITY ASSAYS

Within the different assays that can be performed in order to determine whether or not someone is either LPR, HPR or normal platelet reactivity there is great variability. One assay may determine that a patient has a HPR while other may determine that the same patient may have a normal platelet reactivity and vice versa. It is for that purpose that one must determine a universal method for the platelet reactivity measurement.

One assay that is used consistently is the VerifyNow assay system it uses an optical detection system that measures the aggregation of the platelets while under the influence of fibrinogen-coated beads and platelet agonists. There are a few advantages to using this method including waived point of care testing (POCT), there is no requirement for Whole blood processing and it is a quick and easy test to perform. The downside to this testing is that it is nonflexible, it is a very expensive test and one will have to monitor the antiplatelet therapy. (26)

Another test that should be mentioned is the LTA (light transmission aggregometry). LTA measures the lights transmitted through a sample of aggregated platelets. It is measured with a percentage from 0 through to 100, 0 referring to a low percentage of platelets that have aggregated and a large transmission of light through the sample. Whereas 100% represents the reciprocal, high aggregation of platelets and low transmission of light through the sample. (10-12). This testing is historically the gold standard diagnostic method for platelet aggregation evaluation. There are a few advantages to using this method including that it is a flexible test, it investigates different platelet pathways and this test is sensitive to anti platelet therapy. The disadvantages of this test include that the sample has to be processed manually, there is a high sample volume that is required and that is it time consuming. (26)

The bleeding time must be mentioned in this section as it is used very frequently in clinical medicine. The bleeding time measures the amount of time required for a patient to stop bleeding once bleeding has started. The advantages of this test is that it is an in vivo test, it is quick and simple to perform and that there is no need for whole blood processing. The disadvantages to this test include that it is an invasive test, the test is poorly standardized and the results of the test

itself depend on many variables such as skin thickness, temperature and the skills of the operator. (26)

The last test worth mentioning is the plateletworks testing. The principle behind this test I that it measures the amount of platelets pre- and postactivation in whole blood. The advantages to this test include that it is a point of care (POC) system, it requires minimal sample preparation and it is a quick and easy screening test. The disadvantages to this study include that it is an indirect assay, it requires an adjunctive platelet count and it has scarce data. (26)

Various studies measured the effect of Clopidogrel in HPR and LPR and its resultant MACE (major adverse cardiac events). LTA, VerifyNow, Plateletworks, IMPACT-R and PFA-100 assays were used in low platelet responders and compared to one another to determine each tests sensitivity and specificity. When these tests were completed it was shown that there was a variability in Hazard ratios ranging from 3-36. In order to measure the platelet reactivity while on Clopidogrel the platelet activity should be measured after platelet activation with ADP. The platelet reactivity should be assessed at the level of intracellular signaling pathway. The method of choice is an ADP stimulated assay because it is more specific and more predictive of thrombotic events. Therefore, the assay that is preferred in patients on Clopidogrel is VerifyNow P2Y12. (35)

2.4.USE OF PLATELET REACTIVITY MEASUREMENT

Although there is lacking evidence linking platelet reactivity testing to mortality benefits, there may be a few scenarios in which one might consider performing platelet reactivity testing in order to determine if the patient may need to switch to another agent such as prasugrel or ticagrelor. One may consider platelet testing in a patient that has undergone stent thrombosis despite being adherent to Clopidogrel. Another scenario in which a patient may have their platelet reactivity measured is if the patient is at high risk of thrombosis or if they are undergoing a high risk PCI (bifurcation lesions, left main artery). (24)

2.5.ADVERSE EFFECTS DUE TO HPR/LPR

Clopidogrel is an antiplatelet medication that has a clear indication for patients that have undergone a PCI due to its role in mortality reduction. There is a large patient to patient variability in response to P2Y12-inhibitors and is therefore an important contributor to bleeding or thrombotic events. These bleeding and thrombotic events are a result of either HPR or LPR. It is thought that a HPR would result in thrombotic events due to a reduced activity of Clopidogrel on the platelets. Reciprocally a LPR is thought to result in bleeding events due to an overly active Clopidogrel response on the platelets. Clopidogrel is an antiplatelet medication therefore there is an increase risk of bleeding events in those patients taking Clopidogrel. It is in this section that the adverse events of HPR will be discussed briefly but more of the focus will be on the adverse effects of LPR and the overall bleeding risk associated with LPR in patients that have undergone PCI.

There were various studies that have been done that show an increased risk in thrombotic events with patients that have a HPR. Some of these events include restenosis of the stents after PCI, non fatal myocardial infarct and cardiovascular mortality. There was a study that was performed, the ADAPT-DES that had shown in 8583 participants that HPR alone was a predictor of ST events. In the early stages of HPR on Clopidogrel the HR (hazard ratio) of ST events was 3.00, 95% CI (confidence interval) was 1.39–6.49 and a P (p value) of ¼ 0.005. Meanwhile the one-year risk of ST events was shown to be HR of 2.49, 95% CI of 1.43–4.31, and a P of ¼ 0.001. Within this study it was shown that around 60% of these ST events were related to HPR. Although this study shows a clear increase in ST events due to HPR, HPR should not be used as

a marker for ST events such as troponins or CK-MB. Instead this should determine whether or not platelet function testing would be of use. (16)

The sibbing II study was conducted to determine the resultant effects of HPR and LPR compared to that of patients with normal platelets responses. In this study they divided a total of 2,533 patients into three groups depending on their PRU values. 975 patients (38%) were classified as enhanced responders, below 188, 428 patients (17%) as low responders above 468, and 1,130 patients (45%) as normal responders 189 to 467. While observing the sibbing II study it was noted that both the enhanced and low responders presented with different results compared to that of the normal responders. The low responders had the highest risk of ST (2.8%) while the high responders had the highest risk of bleeding (2.2%). It was shown that the odds ratio was 2.6 when comparing the LPR to those with normal platelet response. (25) With that mentioned it was also determined that there was no evident difference between enhanced responders and normal responders in terms of ST risk. Also there is no evident difference between the bleeding risk in low responders and normal responders. (37)

Also during the sibbing II study it was found that LPR on Clopidogrel was associated with a three-fold higher risk for in-hospital major bleeding events. (25) Several studies have been conducted in order to determine whether platelet reactivity has an influence in bleeding events. In this past study it was uncovered that those that presented with a low platelet reactivity had a three-fold increase in bleeding events compared to those with normal platelet function. In another study called Antiplatelet effects of clopidogrel and bleeding in patients undergoing coronary stent placement that was conducted included more than 8500 participants. Those patients with a PRU value of less than 208 presented with an increased risk of bleeding events. Due to the thrombotic events that take place in patients with HPR on Clopidogrel and bleeding events that occur in those patients on Clopidogrel with LPR, it is important to determine a therapeutic window in order to avoid these adverse effects. (18)

There was another study that was conducted called the ARMYDA-BLEEDS in order to determine if there is an increase in bleeding within the patients that were both on Clopidogrel and have a LPR. The study was conducted on 310 patients that were placed on Clopidogrel post PCI either due to NSTEMI or due to angina with inducible myocardial ischemia. The PRU cut-off values were determined to be at 189 measured by the VerifyNow system. It was shown within this study that the major bleeding events that occurred at 1 month was 11.6% in patients with pre-PCI PRU values below 189 and 1.9% in those with PRU values above 189 (p 0.001). The minor bleeding events that occurred were also elevated in the group with the PRU level below 189, pre-PCI PRU values below 189 (13.7%) compared to those with PRU values above 189 (5.1%) (p 0.001). (33)

There was another study that was conducted, the Campo trial, this trial looked into the relationship between genetic variability of the CYP2C19 enzyme and the impact this would have on Clopidogrel function. This study took 300 patients that had recently had PCI and placed on Clopidogrel and each patient was checked for genetic variability in the CYPC19 enzymes. Of these patients that had genetic variabilities, the adverse effects of Clopidogrel were compared in this group to those that did not have genetic variability. Within this study it was shown that the risk of bleeding with a CYP2C19*17 genotype variability is a HR of 2.3, a 95% CI of 1.03 to 5.3 and a p of 0.03. (38)

There are a few points that are evident through these various studies. Firstly, through both the ARCTIC trial and the ADAPT-DES trial it is shown that there is a clear increase in risk of ST events in patients that have HPR, HR of 3.00 shortly after the introduction of Clopidogrel and a HR of 2.49 after one year. Secondly, through the sibbings II trial it was shown that there is an evident increase is ST events related to those patients with HPR as well as an increase in bleeding events in those with LPR.

Also within the sibbing II trial there was an increase of bleeding events in LPR patients compared to that of patients with normal platelet reactivity with an odds ratio of 2.6. When looking at the hyper response trial it showed an increase in bleeding risk in those patients that presented with a PRU of below 208 when compared to those patients with a PRU of above 208. In the ARMYDA-BLEEDS trial a link was clearly shown between LPR and bleeding events, both minor and major. It was also shown within the campo trial that there is a clear link between the CYPC19 enzyme and bleeding risk. It is through these trials that one can evidently see a link

between LPR and bleeding risk and also that there may be a link between bleeding risk on Clopidogrel with genetic variability of the CYPC19 enzymes.

2.6.ROLE OF GENETIC VARIABILITY

There is a 15-20% genetic variability between people in the general population and this variability results in an over or under activation of Clopidogrel. These genetic variations are apart of the two step oxidation hepatic processes and mainly affect the p450 enzymes within the liver. The main alleles that are encompassed within this group are CYP2C19*17 and CYP2C19*2 and both can have either gain of function or loss of function of these enzymes. (4-6)

Within the Campo study a few variables were measured in regards to Clopidogrel function on platelet reactivity. One of the variables that was looked at was the genetic polymorphisms and how that would affect the platelet reactivity. It was uncovered that the genetic polymorphism accounted for approximately 18% of the platelet reactivity variation and also that there is a risk of bleeding with a CYP2C19*17 genotype variability of HR of 2.3, a 95% CI of 1.03 to 5.3 and a p of 0.03. (38)

In more detail the platelet function test in clinical cardiology quantified the resultant effects of these genetic polymorphisms on platelet function. The accumulation of 9 studies in 9,685 patients that had undergone PCI and in another that involves the accumulation of 10 studies in 11,959 patients had both arrived at the same conclusion. A reduced function of the CYP2C19 alleles with patients treated with Clopidogrel will result in an increased risk of cardiovascular

events. (19,20) On the other side of the spectrum there were 2 recent meta-analyses that were done that had shown that although there was an association between CYP2C19 genotype and Clopidogrel responsiveness, the actual CYP2C19 allele was not associated with cardiovascular events. (19,20). As shown in the previous studies there is conflicting evidence in regards to genetic polymorphism of the CYP2C19 enzyme and cardiovascular events in patients while on Clopidogrel and have underwent PCI. All that is truly known is that there is clearly a link between certain genetic polymorphisms and ischemic events/bleeding risk.

The increase in ischemic events is represented in the platelet function test in clinical cardiology where it was mentioned, HRPR was increased 11-fold in homozygotes and increased 62% in heterozygotes for the CYP2C19*2 gene, when compared to those that do not have that polymorphism. The study also did not find any association of the CYP2C19*17 gain-of-function gene with reduced on-treatment platelet reactivity. (21) It was uncovered that there are a few genetic polymorphisms that patients may possess that ultimately cause adverse effects in those taking Clopidogrel. These genetic polymorphisms include, the two types of CYP2C19 gene which are CYP2C19*2 and *17. In the GIFT (Genotype Information and Functional Testing)

study 40 polymorphisms were tested and cross matched with the platelet reactivity after Clopidogrel use. Of these 40 the CYP2C19 gene was tested and the results were interesting. The CYP2C19*17 yielded no difference between those with this polymorphism and those with normal platelet response. Whereas those patients that have the CYP2C19*2 gene in homozygotes had a 11-fold increase in high platelet response and a 62% increase in heterozygotes compared to non carriers. (22)

2.7.DOSE ADJUSTMENT

Several studies have been conducted to determine whether an increase in Clopidogrel dose would decrease the ischaemic risk in patients with HPR on standard dose of Clopidogrel.

The GRAVITAS study enrolled 5479 patients with 41% being identified by the VerifyNow assay, all of which had undergone PCI for either stable angina or NSTEMI (non ST elevated myocardial infarction). These patients were split up into two categories, one group which would receive high dose Clopidogrel (600 mg loading dose and 150 mg maintenance dose) and another which would receive standard dose (300 mg loading dose and 75 mg maintenance dose). In this study cardiovascular death, myocardial infarction, or stent thrombosis at 6 months were determined. It was shown that there was no observable difference between both groups.

Another trial was conducted observing the relationship between high dose Clopidogrel and standard dose Clopidogrel in ischemic events in the RE-CLOSE-2 ACS trial with 1789 patients

who have undergone PCI and have HPR. It was uncovered as in the GRAVITAS study that the end result was similar between the two groups. (23)

In ARCTIC multicentre randomized trial, the difference between patients that had medication adjustment based on the variation in their platelet reactivity (VerifyNow assay) and those that had medication adjustment at the discretion of their physician's best judgement was observed. This trial was conducted on 2440 patients that had undergone PCI either due to stable angina or due to NSTEMI. There was a pre determined algorithm depending on the results of the platelet reactivity testing which included high-dose clopidogrel, high-dose aspirin, and glycoprotein IIbIIIa inhibitors (GPI). The primary endpoint in this trial that were followed were death, myocardial infarction, stent thrombosis, stroke, or urgent revascularization after 1 year. It was observed that there was no significant difference between the group that had measured their platelet reactivity and those that were given medication at the discretion of the physician.

For now, there are no studies that show a difference between the reduction of Clopidogrel dose below 75 mg/day and a decrease in bleeding risk in patients that have LPR on standard dose (75 mg/day). Also it seems that switching these patients from clopidogrel to ticagrelor or prasugrel would not reduce bleeding complications because these drugs induce even more excessive P2Y12 – receptor inhibition.

3. CONCLUSION

The use of dual antiplatelet therapy with both aspirin and Clopidogrel are important in the reduction of thrombotic events in patients post PCI. Although in the majority of patients there is a reduction in thrombotic events there is a fraction of the population that have resultant adverse effects. About 15-20% of the population have an inherited genetic polymorphism of the CYP2C19 gene which has around an 18% influence on platelet reactivity. If during the use of DAT the patient has LPR it may result in bleeding events, meanwhile an HPR may result in ischemic events. Due to the several studies that prove a link between HPR and ischemic events, alternatives were sought after to avoid these adverse events. ECS now recommends the use of ticagrelor or prasugrel instead of Clopidogrel because it avoids the CYP2C19 metabolism ensuring no resultant HPR. In regards to LPR the situation is less clear. Due to LPR there may be a resultant bleeding event but the aggregation tests are unreliable when determining the value at which a bleeding event may take place. There are various studies providing evidence of increased bleeding risk in LPR and it is evident that there are cut off values which show an indication for increased risk. With the given cut off values one would be able to determine an obvious therapeutic window for the P2Y12 inhibition. In the campo study it showed an association between the CYP2C19*17 genetic polymorphism and an HR of 2.3. Even if a patient is found to have LPR or a genetic polymorphism of CYP2C19*17 currently there is no available solution to reduce this risk of bleeding. For now, there are no studies that show a reduction of bleeding complication after reduction of Clopidogrel dose below 75 mg/day or switching patients to ticagrelor or prasugrel. The only possible strategies are giving bivalirudin instead of heparin, radial approach, routine use of PPI (proton pump inhibitors) and vascular closure devices in the

case of femoral approach. Probably the lack of other measures is the true reason why there are no positive results from large randomized control trials showing a clinical benefit in using aggregation tests to detect LPR.

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- Received Bilingual (English/Croatian) diploma for grade 12
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