Clopidogrel-related platelet inhibition: correlation with peri-operative adverse events in neurointerventional procedures

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Abstract
INTRODUCTION: The measurement of platelet inhibition (PI) level may be useful in quantifying the risk of thromboembolic complication in subjects undergoing endovascular treatment using implantable materials. We studied the predictability of the VerifyNow point-of-care assay in a large sample of consecutive neurointerventional procedures. METHODS: The percentage of P2Y12-inhibition was systematically measured in a total of 271 procedures (245 patients). The incidence of poor response and adverse events within the first 48 hours were recorded. RESULTS: The overall occurrence of poor response after a single loading-dose of clopidogrel of 300 mg was 61.3 % using a cut-off of 40 % and 43.9 % using a cut-off of 20 %. In the analysis of the incidence of adverse events by P2Y12-inhibition grouping, a significant association was observed between thromboembolic events and low response, with an overall incidence of 10.2 % (cut-off of 40 %) and 11.8 % (cut-off of 20 %). The assessment of predictability using different cut-offs showed that more than 90 % of thromboembolic events would be in the group of poor responders using a 40 % cut-off and more than 75 % using 20 %. CONCLUSION: The use of the VerifyNow assay in the neurointerventional context seems a valuable tool in the early detection of individuals at risk of peri-operative thromboembolic adverse events.

Keywords: stents, stroke, platelets, haemorrhage, thrombosis.

Introduction
Antiplatelet agents are routinely administered in patients undergoing neurointerventional procedures utilising implantable material, e.g. intracranial stent placement. Insufficient platelet inhibition (PI) has been strongly associated with an increased risk of thrombus formation and embolic complications [1, 2]. Consequently, patients are pre-operatively prepared with either a loading-dose or a period of antiplatelet treatment. This is supported by both literature to date and previous experience in the cardiology field.
Whilst aspirin resistance seems relatively uncommon, clopidogrel resistance is much more frequent (28 to 66 %) [1-3]. Since thromboembolic adverse events seem highly concentrated in the low responder group [1, 2], a level of at least 40 % of PI has consequently been recommended.

Individual response to clopidogrel may be evaluated using different techniques. Recently, however, point-of-care assays have been commercially available allowing practitioners to perform prompt measurements pre or peri-operatively. The level of PI is now routinely assessed before intracranial stenting in many centres. In selected cases, antiplatelet therapy might be adapted in order to achieve the recommended levels.

Such an approach requires systematic pre-operative blood sampling, subsequent drug administration and introduces additional costs. The study of any potential benefits in achieving a level of anti-aggregation over 40 % in patients undergoing intracranial procedures is imperative, as is the assessment of the risk for haemorrhagic adverse events. In the neurological field, studies to date investigating these issues are limited.

In a sample of 271 neurointerventional procedures, we have retrospectively assessed the incidence of peri-and early post-operative thromboembolic and haemorrhagic events in relation to the clopidogrel-related percentage of anti-aggregation. The purpose of the present study was twofold: a) to examine a possible association between low response as measured with the VerifyNow point-of-care assay and the incidence of thromboembolic and haemorrhagic adverse events in a large neurological sample, and b) to assess the extent to which this association is influenced by the level of anti-aggregation, by analysing the assay results using two different cut-offs of P2Y12 inhibition: 20 % and 40 %.

**Methods**

**Sample**

The patients included in the study fulfilled the following inclusion criteria: aged between 18 and 90 years old and treated with a non-urgent endovascular procedure intended for intracranial or supra-aortal stent placement. A VerifyNow System (Accumetrics, San Diego, California, USA) was used for measuring clopidogrel-related PI in whole blood samples. The timing for antiplatelet effect testing was immediately after intubation for all patients.

A composite comorbidity score was attributed to each patient according to the presence of concomitant diseases at the time of endovascular treatment. A point was given for any of the following five categories: i) past history of arterial hypertension, coronary or peripheral arterial disease; ii) smoking; iii) obesity; iv) metabolic disease (e.g. diabetes mellitus) and v) known hypercoagulable state. The comorbidity score was given as the sum of accumulated points. All data was collected concurrently to treatment.

Additional information on the sample composition is provided in the Supplemental Material.
Endpoint definitions

Thromboembolic

The following critical events were considered as thromboembolic complications for statistical purposes and classified in one (or two) of the categories below.

Central: Partial or complete intra-stent thrombosis, perforating artery occlusion or distal thromboembolism with ischaemic stroke confirmed by MRI.

Peripheral: Thrombosis or embolism at the arterial access site (e.g. femoral artery).

Severe: Any neurological thromboembolic event in which complete occlusion of the target vessel was observed or that resulted in the necessity of ≥72 h of intensive care or death.

Haemorrhagic

Haemorrhagic endpoints were defined as the occurrence of one or more of the following critical events, classified in one (or two) of the categories below.

Central: Any subarachnoid haemorrhage or intracranial haematoma.

Peripheral: Any retroperitoneal, superficial or external bleeding from the arterial access point requiring non-scheduled medical attention, blood derivate transfusion or any surgical or endovascular treatment.

Severe: Any haemorrhagic event necessitating surgical evacuation, external ventricular drainage, or ≥72 h of intensive care or death.

Statistical analysis

Patient and treatment characteristics were presented using mean, SD and range for continuous variables and frequencies as well as proportions for categorical variables. When a single patient was treated for 2 lesions in 2 different procedures, each one was categorised and analysed independently. The incidence of per and post-operative complications within the first 48 h were compared between groups of procedures according to the PI range and in consideration of 4 categories of events: central/haemorrhagic, central/thromboembolic, peripheral/haemorrhagic and peripheral/thromboembolic. The following groups were considered: PIs≤20 %, PI>20 %, PI≤40 %, PI> 40 % and 20 %<PI≤ 40 %. Differences in the incidence of variables between groups were compared using the chi-square test, the Fisher's exact test or the Student t-test according to the specific situation, with a 95 % confidence interval and a level of significance p<0.05 for the whole study. The same was performed for the analysis of severe thromboembolic or severe hemorrhagic endpoints. Two different samples were considered for these analyses: 1) all cases and 2) stented cases.

A poor response to the loading dose of clopidogrel was defined as a level of P2Y12 receptor inhibition inferior or equal to the used cut-off. The correlation of the VerifyNow assay results with the occurrence of thromboembolic complications was further assessed using the Receiver Operating Characteristic (ROC) curve and the area under the curve, considering sensitivity and specificity as predictability for the occurrence or absence of thromboembolic endpoints respectively. The influence of the body weight on the
PI after a loading-dose of clopidogrel were assessed with ANOVA (the Tukey's test) and the Pearson correlation coefficient.

Results

Sample
A total of 271 procedures were performed on 245 patients. All patients were originally scheduled and prepared for intracranial or supra-aortic stent placement. The mean age at treatment was 58±13.9 years old (mean±SD, range 20-88.5).

In a total of 185 (68.3 %) cases at least one stent was deployed. In 76 others a different technique was applied and the patient did not receive a stent and in a further 10 procedures it was not possible to complete the treatment.

Supplemental Table 1 shows demographic characteristics for the 271 procedures and by range of P2Y12-inhibition.

Clopidogrel related PI
Previous use of antiplatelet medication was recorded in 36 cases for clopidogrel and in 53 for acetyl salicylic acid (ASA). In 43 cases, both were taken.

The mean clopidogrel P2Y12 receptor inhibition at the time of the endovascular procedure was 36.4±34 % (mean±SD, range 0-100) for the whole sample, 44.5±34 % (range 0-98) for the subgroup of patients already taking clopidogrel 75 mg daily and 33.1±33.5 % (range 0-100) for the subgroup with no previous use of this medication. During the procedure, the overall prevalence of poor responders was 61.3 % (PI≤40 %) and 43.9 % (PI≤20 %).

The mean P2Y12-inhibition after a single loading-dose of 300 mg in patients with no previous use of clopidogrel was 33.1±33.6 % (n=182, range 0-100). A total of 65.4 % of those cases presented with PI≤40 % and 50 % presented with PI≤20 %. In the subgroup of patients with chronic use of clopidogrel, a total of 51.3 % presented with a P2Y12 ADP receptor inhibition ≤40 % and 31.3 % with 20 % or less.

In order to assess the effect of body weight on PI obtained after a single uniform loading-dose, patients with no previous use of clopidogrel were grouped in 3 categories according to the range of weight (Supplemental Figure 1). The percentage values of P2Y12 receptor blockade was significantly lower in patients with body weight greater than 60 kg. A small correlation was indicated by the Pearson Coefficient, which was -0.2538 (95%CI -0.39/-0.11, p=0.0006).

Incidence of peri-operative thromboembolic and haemorrhagic adverse events
The rate of symptomatic neurological complications (n=12) at 48 h after treatment was 4.4 % for the whole sample, 4 % for cerebral aneurysms and 6.25 % for intracranial or supra-aortic stenosis procedures. The mortality was 0.4 % at 48 h.
According to the previously established criteria, 72 adverse events were recorded. The frequency of the thromboembolic endpoints was in 17 cases central and in 1 peripheral. Regarding the hemorrhagic endpoints, 8 central and 47 peripheral events were observed.

After dividing the sample into 2 groups using PI≤40 % as a cut-off, a statistically significant difference was observed for the incidence of thromboembolic endpoints (p=0.002), which was 10.2 % in the PI≤40 % group and 1.0 % in the PI>40 % group. All events except one were of central type. If 20 % is used as the cut-off, a statistically significant difference was still observed (p=0.003) with incidences of 11.8 % and 2.6 % (Figure 1). The mean P2Y12-inhibition was 13.4 % among patients presenting with thromboembolic endpoints and 38.1 % in the others. No statistically significant difference was observed for haemorrhagic endpoints.

When considering three categories of range for P2Y12-inhibition (PI20%, 20%<PI≤40% and PI>40%) no significant difference among groups was found.

A total of 9 severe endpoints were recorded.

Five were of thromboembolic type:

- An MCA occlusion due to thromboembolism early in the procedure for the treatment of a cerebral aneurysm with stenting and coiling (PI=36 %);
- A fatal vertebrobasilar thrombosis following stent-assisted treatment of a superior cerebellar artery aneurysm in which per-operative aneurysm bleeding occurred (PI=0 per-operatively and 15 % 23 h later);
- Dissection, thrombosis and temporary occlusion of the internal carotid artery during a stent-assisted treatment of a brain aneurysm, resolved with thrombolytics and stenting (PI=11 %);
- A vessel occlusion during stent-assisted treatment of a middle cerebral artery (MCA) aneurysm, resolved after administration of an intravenous bolus (0.25 mg/kg) of abciximab (PI=0);
- Fatal thrombosis and ischaemia in the vertebrobasilar territory after a failed attempt to treat a vertebral artery stenosis in an elderly woman (PI=0).

And 4 were of haemorrhagic type:

- A fatal dissecting hematomahematoma of the sylvian fissure after stenting of the MCA for aneurysm treatment (PI=92 %);
- An intraparenchymal haematoma after angioplasty of a pre-occlusive MCA stenosis (PI=52 %);
- A subarachnoid haemorrhage after treatment of an MCA stenosis with stent and angioplasty (PI=32 %);
- A life-threatening retroperitoneal haematoma (PI=0).
The frequency of each type of endpoints in the whole sample or specifically among intracranial stenting procedures is shown in the Supplemental Material. Ten out of 18 thromboembolic endpoints and 4 out of 5 severe thromboembolic endpoints were recorded in patients presenting with a co-morbidity index ≥2.

Using ROC curve analysis for the thromboembolic endpoints (Supplemental Figure 2), the point closest to the maximal simultaneous sensitivity and specificity possible corresponds to a cut-off of antiaggregation at 15% of P2Y12-inhibition. The area under the curve is 0.735.

Discussion

The present study examined the impact of a poor response to clopidogrel as measured by the VerifyNow point-of-care assay on the incidence of thromboembolic and haemorrhagic peri-operative complications. In a sample of 271 neurointerventional procedures with implantable material, the use of a cut-off of 40% of PI resulted in an overall prevalence of 61.3% of low responders and the incidence of thromboembolic events in this subgroup was 10.2%. With a cut-off of 20% of PI, a total of 43.9% of the patients were considered poor-responders and the incidence of thromboembolic adverse events among low responders increased to 11.8%. A statistically significant difference between good-responders and low-responders was observed using both cut-offs.

Recently it has been possible to measure the individual response to clopidogrel by different assays, such as the VerifyNow, PlateletWorks (Helena Lab., Beaumont, Texas, USA), IMPACT-R (with and without ADP stimulation, DiaMed AG, Cressier sur Morat, Switzerland), DADE PFA collagen/ADP test (Siemens Healthcare Diagnostics Products, Marburg, Germany) and others. Nonetheless, only measurements using light transmittance aggregometry tests (VerifyNow and PlateletWorks) seem to be significantly correlated to the occurrence of ischaemic adverse events in patients undergoing elective coronary stenting [4].

Laboratory light transmission aggregometry is often considered as the gold standard for platelet function testing. However, this method is time consuming and requires an experienced laboratory. A point-of-care assay, such as VerifyNow, has the advantage of the simplicity of its utilisation and fast results. Consequently, there is significant discussion on this topic. When the laboratory measurement is the gold standard, the point-of-care assay has a sensitivity of up to 55% and a specificity of up to 85% [5]. Other authors have observed a greater area under the ROC curve using the point-of-care assay, and suggest that it should be considered superior to traditional laboratory methods that may be operator-dependent [6].

Even though further studies focusing on these questions are still needed, neuroendovascular surgeons are mostly interested on how the levels of PI relates to the incidence of adverse events. The responses to that relevant question may not correspond exactly to the thresholds that have been identified in previous pharmacological studies.

Despite the data accumulated on the impact of such tests on the evolution of coronary disease, our knowledge is still very limited in the neurological field. Even though the level of PI can be easily detected using point-of-care assays, the usefulness in measuring this in daily neurointerventional practice is still not fully understood. A small number of studies have addressed this question, yet little evidence exists on the predictability of aggregometry assays on the operative risk for neurovascular diseases.
Müller-Schunk et al. (2008) [2] have reported a strong association between insufficient PI and peri-operative thromboembolic complications in a sample of 34 patients undergoing stent placement for supra-aortic stenosis, and another 16 for intracranial stenosis. The 5 patients who experienced adverse events of a thromboembolic type were poor responders to clopidogrel. In a subgroup of 36 patients who responded well, no peri-operative thromboembolic complication was observed. The global incidence of antiplatelet resistance in that series was 28%.

The same year, Lee and coworkers [1] reported their experience with 106 patients undergoing neurointerventional procedures, observing a poor response in 42.9 % of patients. Three cases of peri-operative thrombosis were also recorded and occurred in the poor-response group.

**Clopidogrel-related platelet inhibition**

As insufficient platelet inhibition PI has been associated with an increased risk of thrombus formation within the arterial segment exposed to the foreign material, a minimal level of PI has been recommended and been used for identification of patients who are poor responders to clopidogrel. The techniques for measurement and cut-off are variable in neurointerventional literature and the evident corollary of this is that the prevalence of drug resistance may vary accordingly. In the original series by Müller-Shunck [2], the 50th percentile (52 U) of the aggregation amongst a group of healthy individuals was selected for cut-off using the Multiplate Analyzer (Dynabyte Medical, Australia). Prabakaran et al. (2008) and Lee et al. (2008) [1, 3] used the VerifyNow system with a cut-off of 40 % of P2Y12-inhibition, while Pandya et al. (in the same year) used it with a cut-off of 50 % [7]. Those studies reported a prevalence of drug resistance varying from to 28 % to 66 %.

Body weight is a significant influence on the level of P2Y12-inhibition after a loading-dose. Lee et al. have observed a similar phenomenon in their series [1] in which a univariate analysis showed response to be significantly associated with the peripheral platelet count and inversely associated with the patient’s weight. However, a multivariate analysis showed that the weight was the only independent factor affecting response. This is possibly related to pharmacokinetic phenomena such as the variation of the volume of distribution for the active metabolite or to its pharmacodynamics, such as the physiological finding that insulin inhibits platelet aggregation and activation [8], an effect that has been shown to be absent in insulin-resistant obese subjects [9-11]. On this basis, we consider that the adaptation of the loading-dose for high-weight individuals may be justified. In the present study the percentage of PI was not correlated with platelet count.

Clopidogrel is also susceptible to significant pharmacological interactions. It has been suggested that its metabolism to the active form may be impaired with the use of concomitant proton-pump inhibitors such as omeprazole. On the other hand, there is significant discussion on the fact that smoking may have a protective effect against poor response to clopidogrel. A paradoxical effect of smoking on the risk of peri-operative stroke was recently observed in the SAMMPRIS Study (Stenting versus Aggressive Medical Therapy for Intracranial Arterial Stenosis) [12, 13]. Never smokers presented with higher rates of stroke after stenting, a difference that was observed in both univariate and multivariate analysis [14]. These data does not point that smoking has a protective effect per se, but rather that it may accelerate the conversion of clopidogrel to its active form. Clopidogrel is a prodrug; 2 consecutive cytochrome P450-dependent steps convert it to its active metabolite [15]. Cigarette smoking is an inducer of CYP1A2,
which is one of the hepatic enzymes involved in the metabolism of the prodrug. There are also arguments in favor of an influence of the degree of tobacco consumption on the degree of enzymatic activation. In patients using chronic clopidogrel therapy that consume less than a half pack per day, the effect of smoking on ADP-induced aggregation is not observed [15] and in normal volunteers, it is known that the level of CYP1A2 activity increases in function of the “number” of cigarettes smoked per day [16].

**Incidence of peri-operative thromboembolic adverse events**

Considering the previous observations on the variability of the effect of clopidogrel in inducing PI, it seemed crucial to investigate the effect on the incidence of thromboembolic (and hemorrhagic) adverse events among poor responders using different cut-offs in a neurological population. Enough evidence exists to suggest that the use of stents and the treatment of symptomatic supra-aortic or intracranial stenosis are related to a significantly increased risk of ischaemic adverse events [17-28]. The level of 40 % of PI suggested in a previous study using the VerifyNow system [3] was based on cardiology literature [29].

In our team’s experience with intracranial stenting, the combination of a loading-dose of clopidogrel the day before the procedure, a point-of-care PI test in the interventional operating room and a dual antiaggregationanti-aggregation regimen after the endovascular treatment has reduced the incidence of thromboembolic events to roughly 50 %. This has been documented in previous publications [25, 26, 28, 30].

Determining the frontier between adequate anti-aggregation and drug resistance has practical implications for neurointerventionists. It can help determining how vigorous antiplatelet preparation should be, the necessity of adapting doses, the implications for patients who may undergo the administration of supplementary doses and all financial issues regarding drug administration and assay utilisation. Even if point-of-care assays seem useful in measuring how efficient our treatment is, evidence on how much they can (or cannot) avoid risks is absolutely necessary to understand if their routine utilisation is justified.

The assessment of the predictability of the assay in the present study showed that the 40 % cut-off would include 94.4 % of the thromboembolic events in the poor response group and the 20 % cut-off would include 77.7 %. For a practitioner working with a similar population, the use of the lower cut-off would hypothesically increase the frequency of thromboembolic endpoints among good responders from 1.0 % to 2.6 % (Figure 1, Supplemental Table 2).

This observation has direct clinical implications. If a hypothetical candidate for an intervention presents low intermediate levels of P2Y12-inhibition (e.g. 23 %), one may indicate a supplementary loading-dose in order to achieve a PI level over 40 %. This type of study provides information on the degree of additional benefit in terms of procedural safety.

We are assessing the predictability of the VerifyNow assay for events that are multifactorial. In the example of the present study, a possible limitation was the fact that the group with less than 20 % of PI was slightly older, which might have contributed to an increased per-operative risk.

This multifactoriality probably contributes to the finding that the overall risk predictability of such a test is modest, as previously reported [4]. This may be assessed by the study of the form and area under the
ROC curve. Since the point closest to the theoretical gold standard (1 value in the Y axis) is relatively near to the bisector and the area under the curve is no more than 0.735, a fair but not very strong association exists between the test and the final event.

A cut-off of 15% was estimated as being the value providing the maximal simultaneous sensitivity and specificity in the studied population. The implications of such a low level were discussed by Godino et al. (2009) in a study comparing the VerifyNow assay and flow cytometry [31]. The authors also found through ROC curve evaluation that an inhibition ≤15% or an absolute PRU>213 are cut-off values that identify poor response with maximal sensitivity and specificity and likely better reflect the physiological reality. However, we do not advocate that such values are the most appropriate in everyday clinical practice. The choice of cut-off must be based both on clinical evidence and investigations into the “tolerable” risk for a specific type of procedure.

**Incidence of peri-operative haemorrhagic adverse events**

This study did not demonstrate any significant increase in the incidence of intracranial peri-operative haemorrhagic events in good responders. However, it is still worth noting that only 1 of 4 patients who presented with severe haemorrhagic endpoints had a PI under 20%. This patient, who presented with a life-threatening retroperitoneal haematoma, was a poor responder subject who had received a per-operative dose of abciximab following a thromboembolic complication. These facts lead to two interesting points. First, a patient presenting with a higher level of P2Y12-inhibition does not seem to be under a significantly higher risk of peri-operative intracranial haemorrhages. However, in cases when it occurs, this will be potentially severe, since haemostasis will be considerably affected by both efficient platelet anti-aggregation and peri-operative anticoagulants (e.g. heparin). Second, insufficient PI may correlate to an increased risk of per-procedural thrombus formation on implantable materials and also to the consequential per-operative utilisation of rescue drugs such as rtPA or abciximab and their collateral side effects.

Interestingly, a slightly higher incidence of peripheral haemorrhage needing medical attention was observed in the low-responder group when the 20% cut-off was used (p=0.04). However, we consider that this data should be interpreted with caution since patients in this group were heavier, which may have predisposed these subjects to the occurrence of bleeding that was clinically important but difficult to compress.

**Limitations of the present study**

Our study presents limitations. Its design does not allow the assessment of the influence of tobacco consumption or the previous use of proton-pump inhibitors on the prevalence of poor response to clopidogrel. Also, the level of aspirin-related inhibition was not routinely monitored. This could potentially have led to an increased predisposition to thromboembolic adverse events related to dual drug resistance. The resistance to ASA has been reported to be considerably less frequent, ranging from 2.1 to 13% [1-3]. However, it has been suggested that poor responses to clopidogrel are more frequent amongst poor responders to ASA [10, 32].
Future perspectives need to include randomisation and possibly larger, prospective studies with longer follow-up. This may help interventionists in better understanding the correlation between platelet function and the long-term evolution of patients who are treated with neuro-implantable devices.

Conclusions
The use of the light transmittance aggregometry assay VerifyNow in patients undergoing neurointerventional procedures seems a valuable tool in the early detection of individuals at risk of peri-operative thromboembolic adverse events. The study of the predictability of this point-of-care assay suggests that the adoption of a 20% cut-off may detect more than 75% of patients at risk of thromboembolism. A 40% cut-off detected more than 90% of them in the studied population, but such level of PI seems more difficult to obtain after a single 300 mg loading-dose. No significant association was observed between P2Y12-inhibition levels and incidences of intracranial haemorrhage, but no definitive conclusions may be taken on its effect on the severity of those events. The level of clopidogrel-related anti-aggregation after a single loading-dose seems inversely related to body weight.

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Conflict of Interest
AB consults for Stryker Neurovascular, and this study was partially supported by Stryker Neurovascular.

Supplementary Material
The supplementary material is available online at:
http://www.ejmint.org/sites/default/files/attachments/original_article_1437000160.suppl_.pdf
References


**Figure 1** - Incidence of thromboembolic endpoints in function of the measured P2Y12 receptor inhibition at the time of the endovascular procedure, using the VerifyNow point-of-care assay (Accumetrics, San Diego, California, USA) and two different cut-offs: 20% and 40%.

*Candidate for endovascular treatment using intracranial or supra-aortic stenting*

- 300mg loading dose
- VerifyNow point-of-care assay

**Incidence of thromboembolic endpoints (n=271)**

- 11.8%* 2.6%*  p=0.003
- 10.2%* 1.0%*  p=0.002

**Incidence of thromboembolic endpoints, stented patients (n=185)**

- 12.5%* 2.9%*  p=0.011
- 10.6%* 1.4%*  p=0.017