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LETTER TO THE EDITOR

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Evaluation of AggreGuide A-100 for monitoring of antiplatelet therapy

Dual antiplatelet therapy with acetylsalicylic acid (ASA) and a thienopyridine platelet $P2Y_{12}$ ADP receptor antagonist is widely prescribed as secondary prevention in acute coronary syndrome and following percutaneous coronary intervention. Clopidogrel is the most frequently prescribed $P2Y_{12}$ antagonist, though variability in its pharmacokinetic and pharmacodynamic effect is extensively described. It is an orally administered prodrug that needs metabolization in the liver in order to exert its antiplatelet effect. Differences in the rapidity and extent of this effect are partially due to genetic variations in the CYP2C19 enzyme that metabolizes the pro-drug clopidogrel in its active metabolite.[1,2] Other factors that may contribute to the variable anti-platelet effect of clopidogrel are differences in absorption (diet, polymorphisms in the ABCB1 transporter molecule), smoking, drug interactions, inherent variations in platelet function before clopidogrel administration and other patient-related factors (obesity, diabetes mellitus, renal dysfunction, inflammation, age…).[3] Similarly, an unpredictable response of platelets to ASA has been reported and has been related to poor clinical outcome, although described prevalence of aspirin resistance varies from <1% to 57% according to different publications.[4-6]

A recent review of Michelson et al[3] focused on the clinical benefit for monitoring of antiplatelet therapy and found that available evidence is not sufficiently strong to support routine use of laboratory tests for monitoring platelet function in patients with so-called aspirin or clopidogrel resistance. According to the American Heart Association/American College of Cardiology 2011 guideline and the 2014 European Society of Cardiology guideline, testing may be indicated in selected patients at risk for poor clinical outcome after percutaneous coronary stenting.[7,8] Tailored treatment based on laboratory results may be a solution for the wide inter-laboratory variability of response to clopidogrel.[9]

Multiple options exist for monitoring of antiplatelet therapy.[9,10] Light-transmission aggregometry (LTA) is considered the historical diagnostic gold standard.[10,11] It is an adaptable technique as multiple agonists can be added in different concentrations. Yet, this technique is expensive, laborious, time-consuming, needs high volumes of citrate plasma and suffers from high intra- and inter-patient variation. Furthermore, technicians need specialized training, diagnostic cut-offs are uncertain and the method is poorly standardized. Therefore, this technique is mostly restricted to specialized laboratories.[10,11] Different point-of-care (POC) analyzers, which use low-volume whole blood samples, are available as fast and easy-to-use alternatives to LTA. One of these POC platelet aggregometers is the AggreGuide A-100 (Aggredyne, Houston, TX, USA), which uses a laser source for measurement of light scattering. The AggreGuide A-100 is designed to be simple and fast, without need for specialized training, no blood preparation or centrifugation, small blood volume and no reagent handling. A previous study already evaluated the effectiveness of this analyzer to detect aspirin-induced platelet dysfunction at low and high doses of aspirin.[12] It was our aim to evaluate the performance of the AggreGuide A-100 as an alternative less labor-intensive method for monitoring responsiveness of platelets to dual antiplatelet therapy ASA and clopidogrel.

During a 3-year period (December 2014-December 2017), 72 citrated whole blood samples (BD Vacutainer, Becton Dickinson, Plymouth, UK) from patients on long-term dual antiplatelet therapy with ASA (80 mg once daily) and clopidogrel (75 mg once daily) were sent to the laboratory and analyzed within 4 hours. Samples were obtained from patients with intracranial stents who were consulting the department of vascular and interventional radiology in the Ghent University Hospital in Belgium. For these patients, monitoring of antiplatelet therapy was ordered because resistance to antiplatelet therapy was suspected due to re-occurrence of an ischemic event despite the use of ASA and clopidogrel. LTA was performed on Chrono-Log 700 (Chrono-Log, Havertown, PA, USA) according to the North American Consensus Guideline on platelet function testing.[13] Platelet-rich citrate plasma was obtained after centrifugation (10 minutes, 180 g/min) (Hettich Universal 32; DJB Labcare, Buckinghamshire, UK). LTA curves were assessed after addition of 25 μL of the agonists adenosine diphosphate (ADP) (2.5 and 5 μmol/L), collagen (2.5 and 5 μg/mL), ristocetin (0.5 and 1.5 mg/mL), epinephrine (10 μmol/L), arachidonic acid (AA) (1 mmol/L), and thromboxane A2 analogue (1 μmol/L) to 225-μL platelet rich citrate plasma. Light transmission was recorded during 6 minutes and compared to platelet poor plasma. Maximal aggregation and disaggregation of platelets activated with ADP was evaluated for effect of clopidogrel and maximal aggregation and disaggregation of epinephrine and AA was assessed for effect of ASA. For measurable effect of therapy, maximal aggregation for these agonists should be lower than 50% and/or significant disaggregation, arbitrarily defined as greater than 20%, should be present.[10,13] For AggreGuide A-100, Platelet Activity Index (PAI), was measured on citrated whole blood after stimulation of platelets with the agonists thrombin-receptor activating peptide (TRAP) (functions as an internal control), ADP (estimate for response to clopidogrel) and AA (estimate for response to ASA). To each disposable test cartridge, which contained a freeze-dried agonist, 164 μL of whole blood was added. Other agonists could not be assessed since only AA, ADP,

and TRAP cartridges are commercially available. Similar to LTA, the platelet aggregation is measured by the difference in light scattering. For measureable effect of antiplatelet therapy, PAI for ADP and AA should be lower than the reference values, as indicated by the manufacturer, for these agonists (4-9 for ADP and 3-9 for AA). Sensitivity of AggreGuide A-100 compared to LTA was assessed and weighted kappa correlation coefficient was calculated using Medcalc® statistical software (Mariakerke, Belgium).

Additionally, check of reference values was performed according to the CLSI C23-A3 protocol by analysis of 23 healthy volunteers.[14]

TABLE 1 2 × 2 table for assessment of effect of clopidogrel (A) and acetylsalicylic acid (ASA) (B) therapy. Platelet Activity Index for the ADP cartridge (for assessment of clopidogrel; reference values 4-9) and the AA cartridge (for assessment of ASA; reference values 3-9) on AggreGuide A-100 are compared to gold standard light transmission aggregometry (LTA)

		AggreGuide A-100		
		+	−	Total
A				
LTA	+	29	42	71
	−	0	1	1
	Total	29	43	72
B				
LTA	+	35	29	64
	−	3	5	8
	Total	38	34	72

Lot-to-lot variability for AggreGuide A-100 cartridges was assessed by analyzing 15 samples with 2 different lots. This study was approved by the local ethical committee.

Our results show a poor comparability of AggreGuide A-100 to LTA. When manufacturer's reference values were applied, 33% of samples were discordant for both ASA and clopidogrel. 36% of samples were discordant for only one agonist (25% discordant for clopidogrel, 11% discordant for ASA). Mostly falsely normal results were found for AggreGuide A-100: PAI was within reference range for AggreGuide A-100 while for LTA an effect of antiplatelet therapy on platelet aggregation was clearly measurable. Only 31% of samples correlated entirely with LTA. For detection of clopidogrel effect, sensitivity of AggreGuide A-100 was 40% compared to LTA. Weighted kappa correlation coefficient was only 0,019 (95% confidence interval [CI]: −0.018 to 0.056), showing no association between both methods. For ASA, calculated sensitivity was 55% and weighted kappa correlation coefficient was 0,071 (95% CI: −0.082 to 0.224) (Table 1). Specificity could not be assessed since no normal samples were included in the method comparison.

AggreGuide A-100 only assesses a decrease in aggregation. Disaggregation of platelets following maximal aggregation (>50%) after stimulation with one of the agonists is however also a sign of treatment response, although this is not detected by the AggreGuide A-100. This seems to be particularly a problem for evaluation of clopidogrel response. For 24% of samples, only disaggregation after ADP stimulation was observed on LTA without a decrease in maximal aggregation. Consequently, all these samples were falsely normal with AggreGuide A-100, contributing to the low sensitivity for clopidogrel effect.

The difference in sample matrix in both tests (whole blood for AggreGuide A-100, platelet-rich plasma for LTA) could also

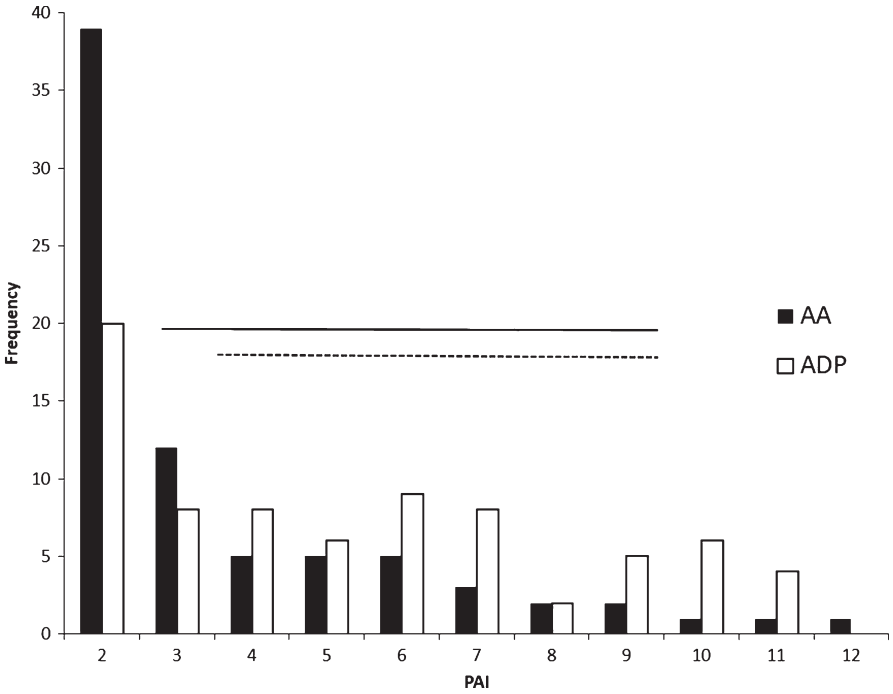

FIGURE 1 Observed Platelet Activity Indices (PAI) of arachidonic acid (AA) and ADP cartridges of patients on dual antiplatelet therapy acetylsalicylic acid and clopidogrel. The bold horizontal line indicates the manufacturer's PAI reference interval for AA, the dotted line indicates the PAI reference interval for ADP

contribute to the poor correlation observed between both methods. Although LTA is still considered the reference method for monitoring of platelet responses,[10,13] some publications state that whole blood platelet impedance lumiaggregometry (WBA) is more sensitive than LTA for monitoring of antiplatelet therapy. Furthermore, since smaller sample volumes are required and specimen processing is easier, WBA may be preferred above LTA. On the other hand, in WBA, whole blood compound such as Von Willebrand Factor, red blood cells, white blood cells, and platelet count may influence the test result.[10,15] For monitoring clopidogrel therapy, vasodilator-stimulated phosphoprotein (VASP) phosphorylation flow cytometric assessment has been proposed as a better alternative to LTA since the phosphorylation status of VASP is a $P2Y_{12}$-receptor-specific method.[16,17]

Figure 1 shows the frequency of the observed PAI's for ADP and AA in the different samples. Our findings differ from what was previously described by Fisher et al[10] They found that the AggreGuide A-100 can effectively measure a decrease in platelet aggregation in patients taking low or high dose aspirin. However, this study was conducted in healthy volunteers taking aspirin instead of real patient samples. Furthermore, the effect of antiplatelet therapy was assessed in a different way. In the publication of Fisher et al, the PAI from samples drawn under therapy was compared to baseline PAI levels before start of therapy, whereas in our study, the observed PAI for samples drawn under therapy was compared to the PAI reference range. Since there were no baseline values before start of treatment for PAI of ADP and AA available in our study, it was not possible to monitor this decrease in PAI after treatment initiation. Overall, a poor standardization between different platelet function tests for monitoring of ASA and clopidogrel is described in the literature.[18-20] This low standardization is one of the reasons why routine monitoring of antiplatelet therapy is not recommended.[3,9]

Check of reference values was successful since <10% of samples from healthy volunteers fell outside specified reference ranges (Figure 2).[14] However, we observed that, in our population, the lower limit for AA could be increased from 3 to 4 since all healthy volunteers had PAI's for AA higher than 4. When a cut-off of 4 instead of 3 is applied for AA, comparison with LTA is slightly better: sensitivity for detection of ASA therapy increases from 55% to 67%. Analogous, percentage of discordant samples decreased from 33% to 24% and percentage of samples concordant for both agonists increased from 25% to 35%.

Lot-to-lot variability was high: for 7/15 samples (47%), interpretation was different with at least one of both agonists. For 2 samples, interpretation was different for clopidogrel, for 3 samples interpretation was different for ASA and for 2 samples, results were discordant for both agonists. Compared to the current lot, observed PAI's for ADP and AA for the new lot were not consistently higher or lower, but discrepant results occurred in both directions.

We conclude that, in this study of 72 patient samples, the AggreGuide A-100 is insufficiently sensitive for monitoring of dual antiplatelet therapy with ASA and clopidogrel and shows poor correlation to light transmission aggregometry. Moreover, lot-to-lot variability is too high to obtain an acceptable reproducibility of results.

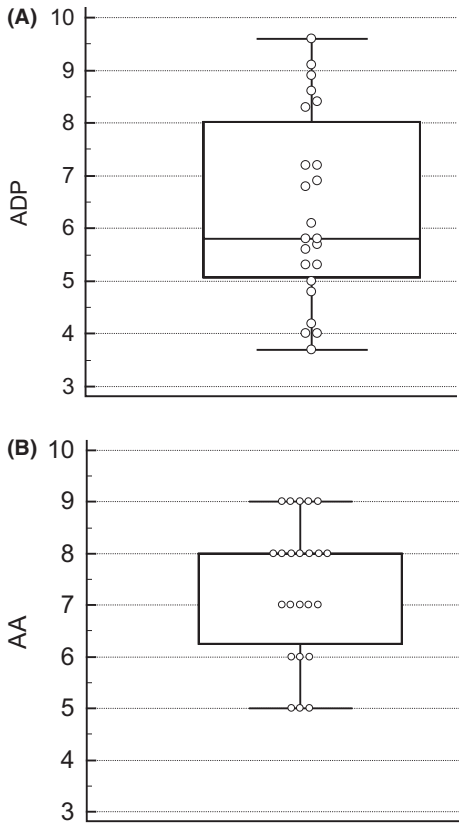

FIGURE 2 Box-and-Whisker plots for Platelet Activity Indices for ADP cartridge (reference values 4-9) (A) and arachidonic acid (AA) cartridge (reference values 3-9) (B), obtained from 23 healthy volunteers

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REFERENCES

1. Harper AR, Price M. Platelet function monitoring and clopidogrel. *Curr Cardiol Rep*. 2013;15:321.
2. Kubica A, Kozinski M, Grzesk G, Fabiszak T, Navarese EP, Goch A. Genetic determinants of platelet response to clopidogrel. *J Thromb Thrombolysis*. 2011;32:459-466.
3. Michelson A, Bhatt D. How I use laboratory monitoring of antiplatelet therapy. *Blood*. 2017;130:713-721.
4. Tantry US, Mahla E, Gurbel PA. Aspirin resistance. *Prog Cardiovasc Dis*. 2009;52:2.
5. Gurbel PA, Bliden KP, DiChiara J, et al. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. *Circulation*. 2007;115:3156-3164.
6. Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thromboelastography platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. *J Am Coll Cardiol*. 2005;46:1705-1709.
7. Levine GN, Bates ER, Blankenship JC, et al. ACCF/AHA/SCAI guideline for percutaneous coronary intervention: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. *J Am Coll Cardiol*. 2011;58:e44-e122.
8. Aradi D, Storey R, Komocsi A, et al. Expert position paper on the role of platelet function testing in patients undergoing percutaneous coronary intervention. *Eur Heart J*. 2014;35:209-215.
9. Cattaneo M. Response variability to clopidogrel: is tailored treatment, based on laboratory testing, the right solution? *J Thromb Haemost*. 2011;10:327-336.
10. Hvas AM, Favaloro EJ. Platelet function analyzed by light transmission aggregometry. *Meth Mol Biol*. 2017;1646:321-331.
11. Cattaneo M. Light Transmission Aggregometry and ATP release for the diagnostic assessment of platelet function. *Semin Thromb Hemost*. 2009;35:158-167.
12. Fisher R, Teitel E, Yee A, Chuang A. Evaluation of the effectiveness of the AggreGuide A-100 in measuring platelet aggregation in individuals taking a high or low dose of aspirin. *Lab Med*. 2013;44:e83-e91.
13. Hayward CPM, Moffat KA, Raby A, et al. Development of North American Consensus Guidelines for medical laboratories that perform and interpret platelet function testing using light transmission aggregometry. *Am J Clin Pathol*. 2010;134:955-963.
14. Clinical and Laboratory Standards Institute (CLSI). *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline*, 3rd edn. Wayne, PA: CLSI: document C28-A3; 2008.
15. McGlasson MS, Fritsma G. Whole blood platelet aggregometry and platelet function testing. *Semin Thromb Hemost*. 2009;35:168-180.
16. Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J Thromb Haemost*. 2005;3:85-92.
17. van Werkum JW, van der Stelt CA, Seesing TH, Ten Berg JM, Hackeng CM. The flow cytometric VASP assay can be used to determine the effectiveness of clopidogrel in patients treated with abciximab. *J Thromb Haemost*. 2007;5:881-883.
18. Helten C, et al. Platelet function testing: dead or alive. *J Thromb Haemost*. 2018;16:984-986.
19. Cuisset T, et al. Clopidogrel response: head-to-head comparison of different platelet assays to identify clopidogrel non responder patients after coronary stenting. *Arch Cardiovasc Dis*. 2010;103:39-45.
20. McGlasson DL, Fritsma GA. Comparison of four laboratory methods to assess aspirin sensitivity. *Blood Coag Fibrin*. 2008;19:120-123.