Platelet function testing (PFT), usually performed by techniques such as light transmission aggregometry (LTA), has traditionally been used to diagnose inherited qualitative and quantitative defects in platelet function, such as von Willebrand disease or Glanzmann thrombasthenia. With the increased use of antiplatelet agents to prevent arterial thrombosis and the interest in identifying patients at risk for thrombosis despite antiplatelet therapy, the use of PFT to monitor the response to and/ortitrate antiplatelet therapy (primarily aspirin and clopidogrel) has become a hot topic. Investigators and practitioners in surgery, critical care, and trauma have also pursued other nonstandard tests of coagulation, such as viscoelastic coagulation testing, to allow more-robust prediction of patients at risk for massive bleeding during invasive procedures or after major trauma. There is considerable controversy regarding the use of PFT and viscoelastic coagulation tests to monitor response to antiplatelet therapy or to guide transfusion decisions for critically ill patients. In this Q&A article, 4 experts in platelet function give their views on both the evidence and current best practice for application of PFT in these nontraditional settings.

What PFTs are used clinically in your institution? What population of patients is being tested, and what clinical decisions are made based on the results?

Gilles Montalescot: We perform VerifyNow (Accumetrics), light-transmission and whole-blood impedance aggregometry and perform some flow cytometric evaluation of platelet reactivity in our research unit for clinical research only; we do not routinely test patients in the catheterization laboratory. Rarely, however, we perform testing on patients when there is a good reason to do so, e.g., assessing compliance with treatment, stent thrombosis, very high-risk patients—all of which are situations that were not evaluated in the ARCTIC (Double Randomization of a Monitoring Adjusted Antiplatelet Treatment versus a Common Antiplatelet Treatment for DES Implantation, and Interruption versus Continuation of Double Antiplatelet Therapy) trial.

Neil Harris: We offer platelet aggregation using a whole-blood impedance assay, as well as PFA-100 testing (Siemens Healthcare) and the VerifyNow. Platelet aggregation is mostly requested by the clinical hematology services (both pediatric and adult). This is commonly in the setting of an individual with unexplained bleeding, in whom all standard coagulation tests are within the reference range.
range. Some of these patients are being worked up for elective surgery. The indications for using the PFA-100 are similar to those outlined for platelet aggregation. The VerifyNow is used by some of our physicians to assess responses to aspirin and Plavix. Over 80% of requests originate in Interventional Neuroradiology and Neurosurgery.

PFT for pediatric patients on the Berlin Heart, a mechanical external pump, has proved very challenging. These patients are typically treated with aspirin and dipyridamole to prevent thrombosis. For a number of years, we have resorted to using the whole-blood impedance aggregometer to guide aspirin therapy in these patients. We have shown that aspirin suppresses the arachidonic acid response and reduces the collagen response by 50%. However, we really have no firm guidelines for assessing aspirin responses in this select group of patients. Dipyridamole testing is even more of a concern, because we are not sure what we are expected to see. The Berlin Heart technical group recommends thromboelastography (TEG) “platelet mapping” to assess the dipyridamole response.

Søren Risom Kristensen:
We primarily use LTA, but we also have a Multiplate (DiaPharma) for whole-blood impedance measurement of aggregation, and a PFA-100. LTA is the technique of choice, but we may use the other methods as well. PFTs are mainly used for investigations of patients with hemorrhagic diathesis to find defects of the platelets. At times, we investigate patients undergoing antiplatelet therapy to determine the effects of the medication, but these are “exceptions,” not the rule.

Matthew Price: I use the VerifyNow P2Y12 test to assess platelet function, usually in patients who have presented with acute coronary syndrome and have been treated with clopidogrel before cardiac catheterization. Observational studies and post hoc analysis of a randomized trial have shown that patients with high on-treatment platelet reactivity are at a greater risk for thrombotic events after percutaneous coronary intervention (including stent thrombosis); therefore, I treat these patients with one of the newer P2Y12 inhibitors, such as ticagrelor. In contradistinction, patients who display a strong antiplatelet effect with clopidogrel (low on-treatment reactivity) have excellent outcomes, and I continue clopidogrel in these patients. Current data would support the contention that such patients may have a less incremental benefit with the more expensive P2Y12 inhibitors, although this has yet to be evaluated in a definitive randomized trial.

How do you define aspirin and clopidogrel response and resistance?

Søren Risom Kristensen: Aspirin resistance is in my opinion “resistance” of cyclooxygenase (COX-1) in the platelets to become acetylated by aspirin; i.e., the platelets will not respond satisfactorily to this treatment. This is a rather rare condition, and it should preferably be measured by a specific COX-1 test such as serum thromboxane B2. LTA with arachidonic acid as agonist is, in my opinion, almost as good as serum thromboxane B2. In some patients, the response of less specific tests may differ from the expected result during aspirin therapy, indicating an “inadequate effect of aspirin” (although COX-1 is inhibited), perhaps because of activation by other routes; this is a situation of low response or high residual platelet activity, which is not a result of resistance.

Since clopidogrel is a prodrug, it must be metabolized in the liver by a cytochrome P450 before platelet inhibition occurs. We know from several studies that various mutations in or polymorphisms of these enzymes result in lowered concentrations of active metabolites, even no metabolites in some patients (resistance). Therefore, the functional tests will show more or less a continuum of response. Low response to clopidogrel is a fairly frequent problem, but a precise discrimination limit between “normal” (i.e., “sufficient”) and low response (also called “high on-treatment platelet reactivity”) is a matter of debate. A consensus paper from 2010 suggested some limits for various methods, including LTA and impedance aggregometry, but, unfortunately, different methodologies do not describe the same group of patients as low responders.

Neil Harris: With respect to whole-blood impedance aggregometry, we established a laboratory reference range by using blood samples from 40–50 volunteers, and any result below the fifth percentile is regarded as abnormal. For aspirin therapy, an expected response is a percentage inhibition of arachidonic acid and collagen-induced aggregation of 50%–60% and 40%–45%, respectively. These guidelines are based on our observations and NOT upon any formal studies. We have not
currently tested or defined responses to clopidogrel (Plavix) using the whole-blood aggregometer.

In the case of our VerifyNow, an adequate aspirin and clopidogrel response is defined as a result of <550 aspirin reaction units and <200 P2Y12 reaction units, respectively. It is important to note that this device was, until recently, able to calculate the clopidogrel “percent inhibition,” but this is no longer available because of a method change introduced by the manufacturer. We do not have a laboratory-based definition for resistance to aspirin and clopidogrel.

**Gilles Montalescot:** Definitions of aspirin and clopidogrel poor response vary from test to test, and sometimes even with the same test; e.g., different cut points have been used with the VerifyNow (P2Y12 reaction units of 208 and 235 for clopidogrel). The thresholds for these tests have been evaluated in large cohorts of patients.

**Matthew Price:** I try to avoid the term “responder” or “resistance.” I define high on-clopidogrel reactivity as >208 P2Y12 reaction units according to the VerifyNow P2Y12 test. I don’t generally test for an aspirin effect, but when or if I do, I define high reactivity as >550 aspirin reaction units, although the data for such a cutoff are not nearly as robust as for the P2Y12 test.

**Patients classified as nonresponders to aspirin and/or clopidogrel by any given test are often found to respond when retested at a later date. Do you think this phenomenon is due to poor reproducibility of tests, variability in treatment response over time, biological variability of platelet function, or some other factor?**

**Matthew Price:** I do not think this is due to poor reproducibility of tests. It is likely due to a host of other factors, including biological variability of platelet function and lack of steady-state levels of inhibition at initial testing.

**Gilles Montalescot:** I would not say this occurs often, but it may happen if (1) your first measurement was made during the acute phase of an acute coronary syndrome and the second measurement several weeks or months later when the patient had stabilized, (2) you have changed the treatment after the first measurement, or (3) you have new factors interfering, such as anemia or inflammation. But, of course, poor reproducibility of tests is also one of the multiple factors.

**Søren Risom Kristensen:** I think that the main reason for different classifications at different time points is problems with compliance. Even when patients are asked about taking the pills, they may deny a problem with compliance; they will not admit it. In our hands, we have not seen any great variability in treatment response over time (if sampling and preanalytical variables have been standardized) with the more specific tests. We do not think that treatment response or biological variability of platelets is a major issue but is of course contributing to different results at different times. However, some of the tests definitely have a poor reproducibility, thereby contributing to the problem.

**Neil Harris:** I believe it to be a combination of many of these factors. Medication is likely central to this. We have encountered patients who are unaware that they are taking aspirin-containing medication. I think we also have much to learn about the effects of polypharmacy, including over-the-counter medication, upon platelet function. In addition to medication, biological factors are no doubt important. von Willebrand factor, a major determinant of platelet function, is an acute-phase protein, and plasma concentrations of von Willebrand factor increase in stressful situations.

**Thus far, large randomized trials of clopidogrel and/or aspirin dosing based on laboratory assessment of platelet function have failed to demonstrate a clear benefit in patient outcomes. Given the lack of positive outcomes in large randomized trials, what do you feel is the role of laboratory testing in guiding antiplatelet therapy today?**

**Søren Risom Kristensen:** I definitely agree that we have no studies documenting that testing the effect of medication on platelets benefits the patients. Therefore, we only rarely use PFTs for this purpose, although we do use it a few times in some patients where we for some reason suspect low response (aspirin resistance of COX-1 has been described, and low response to clopidogrel is not rare). However, most of these patients will simply have their medication changed based on the clinical history, and testing of platelet function will rarely give any definite indication of another therapy. Consequently, without the clinical evidence I do not think that laboratory testing of antiplatelet therapy has any major role in routine clinical care today, but I will not exclude the possibility of implementing it routinely if future clinical studies would indicate benefit for the patients.

**Matthew Price:** I believe one must apply the results of the randomized trials to the patients represented in those trials, while at the same time considering the limitations of the trials that have been performed. The 2 large randomized trials—GRAVITAS (Gauging Responsiveness with a VerifyNow Assay—Impact on Thrombosis and Safety) and ARCTIC—both examined predominantly an elective percutaneous intervention (stable coronary artery disease) population, which
we have learned have very low event rates with newer drug-eluting stents, irrespective of the level of on-clopidogrel platelet reactivity. The ARCTIC study was furthermore limited by 2 things. First, adjustment of antiplatelet therapy using PFT was not mandated by the protocol but was instead at the discretion of the operator. The predominant intervention in those patients in whom maintenance antiplatelet therapy was adjusted was high-dose clopidogrel, which has been shown in prior studies to provide only modest pharmacodynamic effect. And, the end point that drove the results—increase of a single troponin measured 6 h after the procedure—is of unclear clinical importance. At this point, PFT should be reserved for those patients with acute coronary syndrome or undergoing elective percutaneous intervention who are at very high risk of ischemic events.

Gilles Montalescot: At the patient level, PFT is not sensitive or specific enough to guide antiplatelet therapy. Perhaps we will get different results with a different platelet function analyzer, or a combination of tests. Perhaps we need to evaluate a different group of patients at higher risk; perhaps we need a different threshold to more accurately determine which patients are at risk; or perhaps we are measuring at the wrong time point to pull out the patients who are truly at risk and might benefit from further therapy. For now, there should not be a recommendation for routine PFT for coronary disease patients.

Neil Harris: This is the reason why our cardiologists do not use PFT to monitor aspirin and clopidogrel. I have to agree with their approach, and we in the laboratory will not encourage testing that is not supported by the literature. I have already mentioned the active requests for PFT from neurosurgery, trauma, and cardiothoracic surgery, especially in the setting of ventricular assist devices. In some cases, these various clinical groups have been conducting their own research and gathering evidence over the years. In other cases, PFT is discussed at national meetings of these clinical specialists. In general, evidence-based studies for aspirin and clopidogrel testing outside of cardiology are sparse, and there is little guidance for us in the laboratory.

Viscoelastic coagulation tests (such as TEG and ROTEM®) that provide information on both platelet function and coagulation factors have become increasingly popular. What do you view as the advantages, limitations, and optimal use of viscoelastic tests of platelet function?

Neil Harris: TEG has been in place at our institution for a number of years. It was originally introduced by anesthesiology for their cardiac surgery patients and for liver transplantation. There has been growing interest in the TEG amongst many clinicians, particularly the trauma surgeons. In May 2012, we introduced RapidTEG (Haemonetics Corporation) for the latter group. The same group as well as the cardiothoracic surgeons are now also interested in TEG “platelet mapping” for intraoperative and postoperative platelet function assessment. The trauma surgeons are concerned about patients admitted to the trauma service on antiplatelet medications. The cardiothoracic team would like to assess platelet function in patients with implanted ventricular assist devices. There are TEG platelet-mapping guidelines for determining dipyridamole therapy in Berlin Heart cases. The clinical groups interested in TEG platelet mapping did not appear to be familiar with alternative methods, such as VerifyNow. It has become apparent to us in the laboratory that the “air time” given to different testing methodologies in the specialized literature greatly influences their decisions and eclipses evidence-based assessment of the results. Of note, however, the VerifyNow does not give a percentage inhibition for aspirin and clopidogrel. I feel this has made the clinicians somewhat wary of its use. Having said this, I believe one would still be hard-pressed to find firm evidence that TEG platelet mapping is superior to the VerifyNow.

What tests do you believe have the greatest potential to predict the risk of bleeding during invasive procedures among patients on aspirin, clopidogrel, or other antiplatelet agents?

Gilles Montalescot: In ARCTIC, we saw some indication that VerifyNow could detect potential bleeders. All the tests need to be carefully evaluated for this indication, which represents a new avenue for research.

Søren Risom Kristensen: This is a difficult question. We have no real therapeutic windows for these tests; i.e., the low values indicating bleeding are even less well defined than limits indicating low or poor response. A few papers have suggested “lower limits” for this use and made predictions that indicate that it may be possible. If antiplatelet therapy has been paused before an invasive procedure, it will be possible to examine how much of the function of platelets has been reestablished from a functional test and thereby predict bleeding risk. I do not have much experience with this issue, but it may be an option in the future.

Neil Harris: A history of how the patient has responded to prior hemostatic challenges, including surgery, dental extractions, and trauma, is very important. Once a bleeding tendency is clearly established, a sys-
tematic analysis of the hemostatic system is required. This analysis encompasses coagulation, fibrinolysis, and platelet function. I believe that aggregometry together with secretion studies (lumiaggregometry) is the best tool we have, albeit imperfect. These studies should be conducted in a specialized hemostasis laboratory with dedicated, trained, and experienced staff. A detailed medication history is required for all the patients.

Matthew Price: The ADAPT-DES (Assessment of Dual Antiplatelet Therapy with Drug Eluting Stents) trial of more than 8000 patients undergoing percutaneous intervention was presented at the 2013 American College of Cardiology meeting. In this large observational study, low on-treatment reactivity as measured by the VerifyNow P2Y12 test was significantly associated with a higher risk of bleeding.

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