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Novel Uses for Platelet Function Testing in the Clinical Laboratory: Where Are We Now?

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Guest:
Dr. Brad Karon is an Associate Professor of Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, Minnesota.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Platelet function testing has traditionally been used to diagnose inherited qualitative and quantitative defects in platelet function, such as Von Willebrand disease, but with the increased use of anti-platelet agents to prevent arterial thrombosis and the interest in identifying patients at risk for thrombosis despite anti-platelet therapy, the use of platelet function testing to monitory response to anti-platelet therapy, for example, aspirin, has become a hot topic. In the March 2014 issue of *Clinical Chemistry,* a panel of experts explored the question, “Novel Uses for Platelet Function Testing in the Clinical Laboratory: Where Are We Now?”

The moderator for this article was Dr. Brad Karon, an Associate Professor of Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, Minnesota, and Dr. Karon is our guest in this podcast.

Doctor, what are the current controversies surrounding the use of platelet function test to monitor response to anti-platelet agents?

Dr. Brad Karon: I think the controversies currently can be summarized as, what agents to test for? Whom to test? And what to do with the results as well as which test to use?

In terms of what agents to test for, this whole idea of resistance or non-response to anti-platelet agents started many years ago with researchers trying to test for what was then defined as a clinical aspirin resistance. And all of these tests were designed to detect the effects of aspirin on platelet function.

For many years, there were numerous research publications in this field, but it sort of fizzled out on its own because in the last 10 years or so, there has been much greater interest in assessing response to Clopidogrel.

And the reasons, the field has shifted is the reality is that response to aspirin is much more uniform and predictable than is response to Clopidogrel for a number of reasons involving pharmacogenomics and pharmacokinetics of Clopidogrel metabolism and mechanism of action.

So at this point, in terms of what to test for, the current strong level of interest is testing for Clopidogrel effect, but there remains interest in testing for aspirin effect as well as testing for effects of some of the more novel or newer anti-platelet agents.

Going on to whom to test, and that that's sort of the, maybe one of the bigger controversies and I think we will touch on it and may be a few times in this session, but with aspirin resistance testing, the field began with the idea of well, you have to test everyone and see who responds.

With Clopidogrel resistance now there has been actually a few fairly large and well done clinical trials and they've shown that that sort of "test everyone" approach produced negative results.

There was no difference in patient outcomes if you tested everyone about Clopidogrel and took different actions for those who had test results, that appeared to indicate a sub-optimal response to the Clopidogrel on platelet function.

And so now the consensus is moving towards testing for high-risk patients, that is patients who are at high risk for some adverse outcome, rather than testing everybody for Clopidogrel response.

The third question, what to do with the results? It's been established using the number of a different laboratory measures that a significant proportion and usually depending on the study, you see 30%-50% of all patients tested, appear to have somewhat of a sub-optimal response to Clopidogrel depending on what test is used and how the researchers define a sub-optimal response?

The primary limitation to using this test routinely now in clinical use is a lack of good evidence for what exactly should be done when a patient appears to be getting a sub-optimal effect from Clopidogrel.

There are some small data sets collected in the context of some larger clinical trials that suggest for some of these selected high-risk populations, the change in therapy may be beneficial if a lab test indicates a sub-optimal response, and again that's why they field has moved towards testing the high-risk patients.

However, there is still sort of a lack of consensus or a huge pool of evidence that suggest, that changing the anti-platelet therapy, either increasing the dose of Clopidogrel or changing to a different anti-platelet agent is going to benefit patients whose laboratory tests shows the sub-optimal response to Clopidogrel in terms of measuring platelet function. And that's a major limitation to using these tests routinely today.

And then finally under the major controversies is, which test to use? Here there is actually a pretty clear and consistent conclusion between a number of studies and the conclusion unfortunately is depending on which test you pick, you will select for a slightly different set of patients or population of patients who we identified as being in that 30%-50% to have a sub-optimal response.

Experts have tried to account for this phenomenon by defining high on-treatment platelet reactivity by multiple tests, so they can allow the person to pick their test they like and then use these guidelines for definition of high on-treatment platelet reactivity to define what, what the cutoff is for that particular test.

This approach does have some shortcomings in my opinion. First, it is likely that the different platelet function tests are really measuring slightly different aspects of platelet function, and so put it into a more of a conventional chemistry context, you are almost measuring slightly different analytes, you are using slightly different lab tests and yet trying to make the same treatment decision with them. And I think in the end, when the physiologic mechanisms or what these tests are measuring is better understood, that will become clear, but at this point, it really isn't clear how these test differ from each other physiologically.

In the end, only outcome data will really help us to answer the question of which test is most valuable and so we need to keep in mind that different populations of patients and different studies, the study conclusions may suggest a different test be used for the different cut off to make clinical decisions.

The second issue is that for test of platelet functions. It's really difficult if not impossible to separately measure what we normally do in a chemical test, biological variability, the analytic and precision, over time and therefore come up with some relative change value or definition of what percent change in the test represents a change in patient conditions.

Some of these tests do have only modest levels of precision and that further complicates the ability to interpret changes in the given test results for single patient over time.

In general, the test that have become more popular over the last few years are those that tended to in studies, minimize the imprecision issues, which at least helps clinicians interpret changes over time, but it's still a challenge clinicians to interpret for a given patient, a change in platelet function values over time.

Bob Barrett: Well, doctor what are some of the commonly used platelet function tests for assessing response to anti-platelet agents and what are some of the advantages and disadvantages of each test?

Dr. Brad Karon: Well, there are growing number of tests available, FDA approved, and in the research pipeline. The ones I will focus on to answer the question are the tests that were evaluated in recent guidelines for determining or defining high on-treatment platelet reactivity. And those tests were the VerifyNow device; Light Transmission Aggregometry, which is a technique, not a particular manufacturer device; Vast Flow Cytometry, again, more of a technique or measurement, flow cytometric measurement, and the multi-plate whole blood impedance aggregometry which is again another particular vendor specific technology.

In our practice, or in our lab, we have a fair amount of experience with each of these tests and so I can comment on their relative advantages and disadvantages through our experience. Though I should point out that in our practice, we use all of these tests only for ongoing research not in routine clinical use.

The VerifyNow has significant advantages, it's probably the most widely used test in both, in terms of number of research studies and in routine clinical use, and so there is a level of comfort or collective experience in using and interpreting these numbers. And that they are using the device and interpreting the numbers. It’s a whole blood assay and it's relatively simple, could be used in the point of care but it doesn't have user dependent aspects to the testing, like some other platelet function tests do.

And in most studies, it’s one of the more if not the most precise assay, so again it eliminate some of the interpretation issues of how to interpret changes in a given patient over time.

Some of the disadvantages, it's relatively expensive in terms of per test cost, for these reagents or cartridges in this case. The test itself take a whole tube of blood. There is

a special collection tube for this test and it uses the whole tube to do the test. And we've also found, at least in our hands, we tend to get more cartridge or test errors such that we put the tube on and we get an error code and because it used the whole tube we can't repeat the test like we can with some other methods. And therefore you may find you get more sort of errors or samples on which you cannot perform a measurement.
Light Transmission Aggregometry, often called the LTA, is often, in terms of advantages, it's in many research studies used or considered the gold standard for measuring the effect of anti-platelet agents on platelet function. And there is a fair amount of research study showing in terms of picking cutoffs and interpreting Light Transmission Aggregometry, there is the fair amount of research out there.

Some of the disadvantages: LTA, although it has been used in laboratories for decades and decades for detecting qualitative platelet function and disorders, it was never really designed or even particularly well validated as a quantitative test of platelet function, and so that is a relative disadvantage.

It is labor-intensive and in studies and proficiency testing and other similar types of studies shown to have more user variability from site to site and there's pretty objective published data that is less precise than some other methods of platelet function.

Going back to the pro-column, the per test cost test for reagents, even those it is labor-intensive, the per test cost for reagents is quite low.

Vast flow cytometry, we have used this in our recent research study here. In our hands--and I think this is backed up in most---in some well-done studies, it is the single test that by far best differentiates platelet function between healthy volunteers or healthy people with presumably normal platelet function and those taking Clopidogrel.

So again maybe the advantage is, it's probably the best test at discriminating effects of Clopidogrel from normal platelet function.

It actually has a relatively low per test cost for reagents compared to some of the other commonly used tests. And the other advantage potentially, it's the only one of the platelet function test I have talked about in this group, that has a stable sample source and so you could potentially do vast flow cytometry in reference lab or send tests to a reference lab, whereas all the other study tests I will talk

about have to be done very soon after blood draw so that can't really be done in a reference lab environment.

Disadvantages of vast flow cytometry and flow cytometry and so there is a cost and level of expertise and user variability associated with performing flow cytometry. It's not a test you are going to stick in a physician's office lab or even most likely a stat lab.

It will have a much longer turnaround time than any of the other tests I will talk about in this group and it only provides information on ADP induced platelet function, so it would give no information on aspirin affects.

Finally, the multi-plate whole blood impedance aggregometer, aggregometry or device, has some advantages. It's also a whole blood and it's a little bit – well, it's less user intensive and user subjective than LTA or Light Transmission Aggregometry, but maybe a bit more so than VerifyNow. So maybe it falls somewhere in the middle, it has some user dependence and a little more time intensive than VerifyNow but less so than LTA.

It has a lower per test cost than VerifyNow and has been demonstrated in some studies internally here and I think published as well, that it's more precise than LTA and perhaps a bit less precise than VerifyNow.

Disadvantages, there is still relatively less outcome data with multi-plate device and it's only very recently been approved by the FDA for use in US CLIA laboratories.

Bob Barrett: Doctor, different studies have arrived at different cutoffs to define appropriate Clopidogrel response by various lab tests. How does this complicate use and interpretation of these tests?

Dr. Brad Karon: Well, as I mentioned earlier, because each test may be measuring slightly different aspects of platelet function or different parts of how the platelet works and also each study uses a different patient population, and the study designs also are different in terms of what actions are taken in response to these platelet function tests.

This makes generalization of findings from these studies very difficult. The optimal approach, if a lab or practice wants to measure platelet function in the patient population is to try to pick a test with the device that has already been studied in a patient population, that's very close to the one that you are going to use it in, and therefore have a study finding with the patient population and a treatment outcome decision, that's very similar to what you would like to do clinically in your practice.

Bob Barrett: Studies have shown that over time many patients with platelet function values that classify them as non-responders, will later have test results indicating adequate response, and patients with platelet function test indicate adequate response will later produce results indicating non-response.

How does this complicate use and interpretation of these tests?

Dr. Brad Karon: Well, in a laboratory, especially within the discipline of clinical chemistry we are used to classifying and measuring changes in test values or variation in test results, as either being due to analytic variability, or we measure this as precision or biologic permeability.

Changes which are greater than that which can be accounted for the combination of analytic and biologic variability are thus assumed to be changes in patient condition and we calculate and we use this Relative Change Value (RCV) as a measure of how much change in the test represents change in patient condition.

For platelet function, each time that blood is drawn, platelets will be activated and because there is going to be a different extent of platelet activation with each blood draw, that makes separately measuring biologic and analytic variation over time extremely difficult, perhaps even impossible.

That means what when one individual patient results change over time there really isn't a great way to know if this represents analytic variability in the test platform, biologic variability in the test, or some true change in patient condition that has made the anti-platelet therapy more or less effective.

The only way I can see around this conundrum appears to be to identify through prospective research studies, the single best time or the best times to measure platelet function and not rely on serial measurements. The best time to measure platelet function would be those times where in the context of well-designed research studies, platelet function measurement with a given device best predicts the desired patient or disease outcome intended to be measured.

So essentially, the way around variability is to use the research to identify when platelet function should be measured and to measure at that time.

Bob Barrett: Well finally doctor, why are nonconventional coagulation assays, such Viscoelastic Coagulation tests, being increasingly used or requested in critical care areas?

Dr. Brad Karon: Viscoelastic Coagulation testing began appearing with regularity in the medical literature in the 1980s. As the groups studying the coagulapathies that developed during liver transplantation began to show that these tests may better guide transfusion therapy, predict coagulopathy better than do some conventional tests of coagulation function in the laboratory.

From the 1990s on, the research was expanded into the area of cardiovascular surgery, and groups showed that the transfusion algorithms that were guided by Viscoelastic testing were perhaps more effective than those algorithms guided by conventional lab coagulation tests.

Although, it should be noted that other studies showed really no difference between transfusion algorithms guided by conventional versus point of care or versus Viscoelastic Coagulation tests.

Finally, in the last 10 years or so, more studies in the trauma field and the field of trauma literature have shown that Viscoelastic tests better detect coagulopathies that developed early in some cases of trauma, especially cases of severe penetrating injuries.

I believe that the increased popularity of Viscoelastic Coagulation testing in the last five or so years is primarily due to the rapid appearance of more and more literature in the trauma field showing that Viscoelastic tests are effective in early identification of coagulopathy among trauma patients, as well as and perhaps more importantly, because hospitals are trying to reduce blood transfusions in order to both reduced costs associated with transfusion but also in response to evidence that blood product transfusion may have more adverse effects than previously thought.

And I think it is probably largely driven by hospitals trying to improve the effectiveness and utilization of blood products and the increased amount of research studies in trauma and other critical care areas showing the value of Viscoelastic testing, has really increased the interest in this area in the last few years.

Bob Barrett: Dr. Brad Karon is an Associate Professor of Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, Minnesota. He has been our guest in this podcast on platelet function testing from *Clinical Chemistry*.

I am Bob Barrett, thanks for listening.