Measurement of the Antiplatelet Effects of Aspirin in Cerebrovascular Disease

To the Editor:

We read with interest the recent article on the use of the PFA-100 in measuring the ex vivo response to aspirin therapy in patients with cerebrovascular disease.1 Most patients were assessed in the acute phase after ischemic stroke or transient ischemic attack, although some patients with asymptomatic stenosis of an extracranial or intracranial artery were also studied. Thirty seven per cent of patients overall were “aspirin resistant” using the PFA-100, and the rate of aspirin resistance was higher in patients receiving 81 mg (56%) than in those receiving 325 mg daily (28%). However there are a number of issues that warrant further discussion.

The authors report that the “PFA-100 device uses a flow cytometry paradigm to determine the ability of whole blood to close an aperture after stimulation using collagen and epinephrine.”1 However, it must be emphasized to the readers that there are significant differences between flow cytometry techniques and the methodology used in the PFA-100 test system. Flow cytometry is a method used for sensing cells, including platelets, as they flow in a liquid stream through a laser beam.2 Platelets in either whole blood, platelet-rich plasma, or after separation from plasma (washed platelets),3 may be injected through a flow cell, and aligned in single file using a transparent and nonfluorescent “sheath fluid” that exerts pressure on the cell suspension being studied. When a platelet passes through the laser beam, light is scattered in different directions and platelets can be identified by their light scatter characteristics. The sample may also be labeled with fluorochrome-linked monoclonal antibodies specific for certain platelet antigens on the cell surface, or stained with specific fluorescent dyes. The degree of fluorescence of the sample can subsequently be used to quantify the expression of the activation marker of interest, and hence the activation status of the platelets in the sample. In addition, one can assess the expression of certain platelet activation markers before and after the addition of a specific platelet agonist in vitro to measure platelet reactivity and platelet function in circulating blood.4

On the other hand, during the PFA-100 test, a whole blood sample is aspirated at a moderately high shear rate (5000 to 6000 s⁻¹) through a 200 μm capillary to a nitrocellulose membrane with a central 147 μm aperture. The sample is not suspended in sheath fluid. The membrane is coated with collagen in combination with either ADP (C-ADP cartridge) or epinephrine bitartrate (C-EPI cartridge).5 The combination of high shear stress and biochemical stimulation activates the platelets and they adhere to the membrane and aggregate to one another, thus forming a platelet plug that ultimately occludes the aperture. The time taken to occlude the aperture is called the closure time and this provides a measure of platelet function in the sample. However, the device does not directly assess platelet activation status in the sample.

The authors included in their analysis an unspecified proportion of patients who were taking nonsteroidal antiinflammatory drugs (NSAIDs) or cyclooxygenase-2 inhibitors in combination with aspirin, because they wanted “to evaluate aspirin responsiveness in routine clinical practice.”5 This is a very important issue that was mentioned in the discussion.1 Although it has been shown that the administration of ibuprofen before aspirin may interfere with the ability of aspirin to maximally inhibit cyclo-oxygenase-1 in platelets, other NSAIDs such as diclofenac do not appear to have a similar effect.6 We agree with the authors’ suggestion that the use of ibuprofen in combination with aspirin could contribute to the phenomenon of aspirin resistance using the PFA-100. However, in theory, the use of a reversible cyclooxygenase-1 inhibitor (eg, diclofenac) in combination with an irreversible cyclooxygenase-1 inhibitor (aspirin) could also enhance the antiplatelet effects of aspirin and prolong the C-EPI closure time to a greater degree than that seen with either agent alone. Because of the potential confounding effects of concomitant medications on the results of the PFA-100, we would like to know the prevalence of aspirin resistance in patients who were on aspirin monotherapy. Furthermore, because it is possible that upregulation of cyclooxygenase-2 in platelets may contribute to the phenomenon of aspirin resistance,7 did the authors observe any patients who were aspirin resistant on aspirin monotherapy and subsequently had significant prolongation of their C-EPI closure times after the addition of a cyclooxygenase-2 inhibitor to their treatment regimen?

The maximum closure time recorded by the PFA-100 is 300 s. In our experience, many patients on aspirin have prolonged C-EPI closure times greater than 300 s. In the results section, Alberts et al compared mean C-EPI closure times between patients on different aspirin doses and formulations. However, if a proportion of patients in their study also had C-EPI closure times >300 s, one can never assume that the data are normally distributed. Therefore, it is more appropriate to use nonparametric statistics to compare median closure times between groups. Do the results differ if nonparametric statistics are used? In addition, if one cannot assume that the PFA-100 data are normally distributed, do the authors consider it justified to use linear regression analysis to investigate the effect of age, aspirin dose, and preparation on the results obtained?

Finally, the authors implied that many patients who were aspirin resistant on the PFA-100 exhibited a “therapeutic effect” when the aspirin dose or formulation were changed. However, they stated that “we do not yet know how PFA-100 results correlate with clinical events such as stroke, myocardial infarction, and vascular death.” Despite the potential usefulness of this device in monitoring and predicting the response to aspirin, should one await data regarding its “clinical usefulness” before justifying the use of higher aspirin doses (>325 mg daily) to achieve an ex vivo response on the PFA-100?

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