Platelet Function Testing in Patients with Acute Ischemic Stroke: An Observational Study

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Background: The measurement of platelet reactivity in patients with stroke undergoing antiplatelet therapies is not commonly performed in clinical practice. We assessed the prevalence of therapy responsiveness in patients with stroke and further investigated differences between patients on prevention therapy at stroke onset and patients naive to antiplatelet medications. We also sought differences in responsiveness between etiological subtypes and correlations between Clopidogrel responsiveness and genetic polymorphisms. *Methods:* A total of 624 stroke patients on antiplatelet therapy were included. Two different groups were identified: "non-naive patients", and "naive patients". Platelet function was measured with multiple electrode aggregometry, and genotyping assays were used to determine *CYP2C19* polymorphisms. *Results:* Aspirin (ASA) responsiveness was significantly more frequent in naive patients compared with non-naive patients (94.9% versus 82.6%, $P < .0010$). A better responsiveness to ASA compared with Clopidogrel or combination therapy was found in the entire population ($P < .0010$), in non-naive patients ($P < .0253$), and in naive patients ($P < .0010$). Multivariate analysis revealed a strong effect of Clopidogrel as a possible "risk factor" for unresponsiveness (odds ratio 3.652, $P < .0001$). No difference between etiological subgroups and no correlations between responsiveness and *CYP2C19* polymorphisms were found. *Conclusion:* In our opinion, platelet function testing could be potentially useful in monitoring the biological effect of antiplatelet agents. A substantial proportion of patients with stroke on ASA were "resistant", and the treatment with

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Clopidogrel was accompanied by even higher rates of unresponsiveness. Longitudinal studies are needed to assess whether aggregometry might supply individualized prognostic information and whether it can be considered a valid tool for future prevention strategies. **Key Words:** Ischemic stroke—platelet inhibitor—secondary prevention—aggregometry.

Introduction

Control of cerebrovascular risk factors and therapeutic secondary prevention are fundamental to prevent ischemic cerebrovascular events (transient ischemic attack [TIA]/stroke). Treatment of noncardiogenic strokes (atherosclerotic, lacunar, or cryptogenic infarcts) is based on antiplatelet agents, which reduce the relative risk of stroke or death on average by about 22%.[1,2] Regarding their mechanism of action, antiplatelet agents are classified in 3 groups: thromboxane inhibitors (Aspirin™ [ASA, Bayer AG, 51368 Leverkusen, Germany] ASA-dipyridamole), Adenosine diphosphate (ADP) receptor antagonists (thienopyridines: Clopidogrel [Plavix®, Bristol-Myers Squibb, New York, NY], Ticlopidine [Tiklid ®, Sanofi SpA, Milano, Italy]), and glycoprotein IIb/IIIa inhibitors. Oral antiplatelet therapy, with ASA, ASA-dipyridamole, and thienopyridines, showing similar effectiveness,[3] is strongly recommended in secondary stroke prevention.[4,5] The treatment of recurrences remains less clear; it is mandatory to exclude alternative causes of stroke and to improve control of risk factors, but no clear guidelines for alternative therapeutic strategies are available. In everyday clinical practice, after recurrence of ischemic stroke, a therapeutic shift to another antiplatelet agent is the most common option, although there is no evidence based on clinical trials indicating that this is associated with a reduction of the risk of recurrence.

In recent years, the clinical response variability to ASA or Clopidogrel treatment and the phenomenon of "low" or "nonresponsiveness", "antiplatelet resistance", or "high on-treatment platelet reactivity" (HTPR), defined as biochemical failure of the antiplatelet agent to inhibit tests of platelet function ex vivo, have been widely explored.[6,7] The mechanisms for resistance might include an insufficient dose, poor compliance, related gene polymorphisms, baseline platelet hyperactivity, and accelerated platelet turnover.[8,9]

Different methods of platelet function testing are available to assess inhibition of function induced by antiplatelet agents: light transmission aggregometry, the gold standard for monitoring antiplatelet effects ex vivo, but with difficult routine application; vasodilator-stimulated phosphoprotein assay, specific for evaluation of Clopidogrel responsiveness; Impact-R Cone and Plate(Let) Analyzer (CPA) (DiaMed, Cressier, Switzerland); Platelet Function Assay-100 (PFA-100, Dade-Behring, Marburg, Germany); and VerifyNow System® (Accriva Diagnostics, Inc., San Diego, CA). A fairly new generation of impedance aggregometer, named multiple electrode platelet aggregometry (MEA; Multiplate, Roche Diagnostics International Ltd, Rotkreutz, Switzerland),[10,11] showed correlations with the estimates of Clopidogrel and ASA antiplatelet effect obtained by other methods.[12]

A greater risk of recurrence of cardiovascular events has been demonstrated in patients with resistance to ASA or Clopidogrel.[13-17] Furthermore, pharmacological interactions with other drugs (e.g., proton-pump inhibitor) have been associated with a diminished pharmacodynamic response to Clopidogrel.[18] Recently, a systematic review and meta-analysis of randomized clinical trials was performed to evaluate the clinical efficacy and safety of intensified antiplatelet therapy versus Clopidogrel at a standard dosage, on the basis of platelet reactivity testing, in patients undergoing percutaneous coronary intervention who presented HTPR.[19] The intensified therapy protocol was associated with a significant reduction in cardiovascular mortality, stent thrombosis, and myocardial infarction, with no difference in the rate of major bleeding between the 2 groups, although the net clinical benefit significantly depended on the risk of stent thrombosis with standard Clopidogrel dose. Similarly, another systematic review and meta-analysis on randomized trials, concerning tailored antiplatelet therapy in antiplatelet-resistant patients, showed a minor occurrence of death or clinical adverse events in personalized antiplatelet therapy compared with conventional treatment.[20]

Although several data are available on cardiovascular diseases, there are a small number of studies regarding monitoring of antiplatelet therapy in patients with ischemic stroke. In most of them, the evaluation of antiplatelet effect has been performed mainly with Platelet Function Assay-100, with evidence of a low responsiveness to low-dose or enteric-coated ASA in a significant proportion of patients (37%) in Alberts et al's study.[21] Other data pointed to limitations of platelet aggregation monitoring, particularly in terms of reliability of results.[22-24] The Trinity Antiplatelet Responsiveness study investigated the prevalence of ex vivo nonresponsiveness in patients with ischemic stroke/patients with TIA evaluated with a "longitudinal definition of HTPR" by comparing responsiveness to antiplatelet therapy at follow-up with patients' baseline values.[25] Payne et al reported a significant clinical impact of monitoring the intake of a single dose of Clopidogrel with flow cytometry and aggregometry before carotid endarterectomy to reduce postoperative embolization.[26] Recently, some studies evaluated