Dear Sir,

The wide interindividual variability of the capacity of clopidogrel to inhibit platelet functions and its possible association with an increased risk of atherothrombotic or haemorrhagic events highlight the importance of performing clinical trials to confirm the link between clopidogrel responsiveness and clinical outcome (1, 2). However, most of the ex vivo platelet function tests used to evaluate the efficacy of clopidogrel therapy, including platelet aggregometry and flow cytometric platelet activation tests, require specialized equipment and rapid processing of the blood samples (less than 2 hours), which limits their applicability in clinical trials. Analysis of the VAsodilator Stimulated Phosphoprotein Phosphorylation (VASP-P) state has been proposed as an ex vivo test to evaluate the pharmacological effect of clopidogrel on platelets (3, 4). This assay has demonstrated its high sensitivity and specificity for clopidogrel treatment in patients with ischemic cardiovascular diseases and its good correlation with the platelet aggregation measured within 2 hours (5, 6). One advantage of this test is the stability of the blood samples (over 24 hours) (6). Nevertheless, in multicentre clinical trials requiring use of a central laboratory, the effects of transport on the samples (shaking, temperature variations) are unknown. The aim of this study was to investigate the stability of blood samples for VASP-P analysis by comparing the results obtained before and after road and air transport for 24 or 48 h.

Twelve patients (6 men, 6 women; mean age ± SD: 67.6±13.0 years) with a personal history of coronary artery disease were recruited in November 2004. Six, including 3 women, were receiving clopidogrel at 75 mg/day. Venous blood samples were drawn from each patient into 5 separated paediatric tubes (BD Vacutainer® NC 0.129M 1.8 ml, Becton Dickinson, Franklin Lakes, NJ, USA). Four tubes were shipped by specific transport and returned to the laboratory while the fifth was kept in the lab.

Figure 1: Platelet reactivity index (PRI) of the 48 transported tubes before (start value) and after (arrival value) road or road and air transport for 24 h (open circles; n=24) or 48 h (closed circles; n=24). Broken line: equality between start and arrival values. ICC: intraclass correlation coefficient [95% confidence interval].

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oratory at room temperature. Four types of routing were tested: i) 24 h, road only (9 h travel, 15 h warehousing), ii) 48 h, road only (20 h travel, 28 h warehousing), iii) 24 h, road and air transport (2 h flight, 10 h road travel, 12 h warehousing) and iv) 48 h, road and air transport (2 h flight, 10 h road travel, 36 h warehousing). The samples were stored in isothermal boxes during transport and the temperature was recorded every 30 min (Thermo-Tracer®, Oceasoft, Montpellier, France). VASP-P was determined in each tube before (start value) and after travel (arrival value) using a standardized flow cytometric assay (Platelet VASP®, Biocytex/Stago, Asnières, France). The results were expressed as a platelet reactivity index (PRI) ranging from 0 to 100% (6) and the reproducibility of the PRI before and after travel was estimated by calculation of the intraclass correlation coefficient (ICC).

The PRI values at start and arrival were strongly equivalent whatever the mode or duration of transport (ICC [95% confidence interval]: 0.962 [0.913–0.988]; p<0.0001; n=48) (Fig. 1) due to the remarkable stability of the fluorescence intensities (data not shown). Similarly, the blood samples kept in the laboratory showed a high stability for the PRI determination at 24 and 48 h (ICC: 0.926 [0.907–0.988]; p<0.0001; n=12). The starting values of the PRI showed a significant difference between patients treated with or without clopidogrel (respectively 60.1±15.4 and 85.5±4.5 %) and this difference was maintained on return of the samples after 24 or 48 h, whatever the routing (p<0.001; ANOVA for repeated values). The mean temperature recorded during transport was 12.8°C (min: 7.0°C; max: 18.3°C) and there was no difference in temperature between the different routes.

This study demonstrates the high stability during transport and storage at room temperature of blood samples for quantitative flow cytometric analysis of VASP-P. In addition, this stability during storage was associated with a persistence of platelet sensitivity to in vitro inhibition by AR-C69931MX, a direct P2Y12 antagonist (data not shown). The reason why VASP-P is so stable as compared to other platelet function tests, such as aggregation or exposure of P-selectin or activated GPIIb/IIIa, is not yet clear and will require further studies. Notwithstanding, this stability should allow us to simplify the logistics in multicentre clinical trials to assess ex vivo the antiplatelet efficacy of clopidogrel or new thienopyridines, or even in clinical practice which might require individualization of the antiplatelet therapy according to the clopidogrel responsiveness (1). Other studies in patients treated with clopidogrel are now needed to determine the predictive value of the VASP-P assay for an adverse clinical outcome.

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