Are P2Y12 reaction unit (PRU) and % inhibition index equivalent for the expression of P2Y12 inhibition by the VerifyNow® assay? Role of haematocrit and haemoglobin levels

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Summary
The results of the whole blood VerifyNow® P2Y12 assay can be expressed as platelet reaction units (PRU) or % inhibition index (%inh), but an optimal cut-off for the assessment of high on-treatment platelet reactivity (HPR) predictive of clinical events has been validated only for PRU. The aim of the study was to study the influence of haematological variables, such as platelet and leukocyte counts or haematocrit / haemoglobin, within the limits indicated by the manufacturer for assay validity, on the results of the test. We performed a comparison of PRU and %inh in a series 186 samples obtained from a clinical trial on patients under dual antiplatelet therapy. The results show that PRU significantly decreases with increasing haematocrit / haemoglobin, whereas %inh does not, due to a parallel change in PRU and iso-TRAP baseline value. PRU and % inhibition index are not equivalent for the definition of HPR, because of their different sensitivities to haematocrit / haemoglobin.

Keywords
VerifyNow® P2Y12, assay, haematocrit, haemoglobin

The evaluation of high on-treatment residual platelet reactivity (HPR) is an emerging issue in interventional cardiology. The VerifyNow® system (Accumetrics, San Diego, CA, USA) measures the agglutination of fibrinogen-coated beads to stimulated platelets in citrated whole blood. In the P2Y12 cartridge, designed to assess responsiveness to thienopyridines, adenosine diphosphate (ADP) is used as agonist, in combination with prostaglandin E1 to limit the contribution of P2Y1. The increase in light transmittance, proportional to platelet aggregation (i.e. inversely proportional to uninhibited platelets), is reported in P2Y12 reaction units (PRU). A second channel contains the thrombin receptor activating agonist (iso-TRAP) as sole agonist, which provides a baseline value (BASE) for platelet function independently of P2Y12, although BASE is slightly reduced under treatment by thienopyridines (1). From these values, a percentage inhibition index (%inh) can be calculated as [(1– PRU/BASE) x 100], which is correlated to the percentage inhibition measured by optical aggregometry using the value found in drug-free patients as the baseline (1). An optimal cut-off value for predicting clinical events, around 235–240, has so far been established only for PRU (2–5). HPR on clopidogrel (HPRADP) is defined by a PRU above cut-off. In the acetylsalicylic acid (ASA) cartridge, designed to monitor the response to aspirin, arachidonic acid (AA) is used as the agonist and the results are expressed as aspirin reaction units (ARU), with a cut-off value of 550 for HPRASA (6).

As a whole blood assay, VerifyNow® can be influenced by variables such as platelet or leukocyte counts, as well as haemoglobin (Hb) or haematocrit (Ht). Little is known about the effect of these variables on the assay performance within the limits indicated by the manufacturer for its validity. To address this question, we took the opportunity of a clinical trial primarily designed to assess HPR on dual antiplatelet therapy in patients > 75 years (n = 93, mean age: 80 ± 4 years) with the VerifyNow® system (7).

The tests were performed 24–48 hours (h) post-percutaneous coronary intervention (PCI) (T1) after a loading dose of 300 mg of clopidogrel + aspirin, and 5–7 weeks later (T2) under maintenance treatment with 75 mg clopidogrel + low-dose aspirin. The main result of this study (7) was the high incidence (68 %) of elderly patients with biological resistance to clopidogrel (PRU ≥235) on maintenance therapy, whereas biological resistance to aspirin (ARU≥550) was rare (10 % and 14 % at T1 and T2, respectively).

No correlation was found at any time between any of the clinical (7) or haematological variables (XE2100®, Sysmex SAS, Roissy, France) and ARU, or between platelet or leukocyte counts and PRU. PRU correlated with %inh (Spearman rank correlation coefficient: −0.93 at T1 and −0.86 at T2, p<0.0001 for each), whereas BASE did not (not shown). By linear regression (not shown), a PRU value of 235 corresponded to 27 %inh and 35 %inh at T1 and T2, respectively. As reported by others (5, 8), HPRADP defined using PRU, was associated with lower Hb (p=0.032 at T1 and 0.030 at T2).
and Ht (p=0.095 at T1 and p=0.012 at T2) but, surprisingly, HPRADP estimated using %inh cut-offs was not associated with lower Ht and Hb.

To explore this discrepancy, we analysed the distribution of the VerifyNow® parameters according to Ht and Hb quartiles (pooled data of T1 and T2). Trends from the first to the fourth quartile were calculated using the SAS (SAS Institute, Cary, NC, USA) “Mixed” procedure to model repeated measurements, as two measurement times were considered for each patient. The mixed procedure incorporates correlations for measurements of the same person. No significant interaction was found between ARU, PRU, %inh and measurement time.

The results are illustrated in Figure 1. ARU was not influenced by Ht or Hb (Fig. 1A), as previously reported (8). PRU (mean±SD: 232 ± 95, min-max: 8–459) tended to decrease as Ht or Hb increased (Fig. 1). BASE, obtained with iso-TRAP as agonist, (mean ± SD: 334 ± 55, min-max: 184–447) also decreased as Ht or Hb increased (Fig. 1B) (p<0.0001). Eisenberg et al. (8) were the first to report that patients with lower Ht or Hb were more likely to have a high BASE value. As BASE varied in parallel with PRU, the PRU/BASE ratio (mean ± SD: 0.69 ± 0.26) was independent of Ht or Hb (not shown). It followed that the %inh, calculated by using the PRU/BASE ratio, did not vary with Ht or Hb (Fig. 1D). Accordingly, the %inh corresponding to a given PRU value would change with Ht or Hb. This is illustrated in Figure 1E for Ht. The magnitude of this effect was surprising. Between the 1st (Q1) and the 4th (Q4) Ht quartiles, %inh corresponding to PRU 235 varied (p<0.0001) from 38% to 24% on pooled data. The same trends were observed with Hb, or when the samples at T1, early post-PCI (37% to 21% between Q1 and Q4) or at T2, on maintenance therapy (40% to 27%), (all changes <0.001) were considered separately.

A practical consequence of our observation is that %inh cannot be substituted for PRU which, so far, has been the preferred mode of expression of the VerifyNow® P2Y12 assay in all published clinical trials linking HPR to post-PCI adverse clinical events. Although it is interesting to note that, in contrast with PRU, %inh is independent of the haematological variables, only a clinical trial would be able to determine which, of PRU or %inh, is the most predictive of clinical events. In conclusion, we have shown that PRU and %inh are not equivalent for the definition of HPR ADP, because of their different sensitivities to Ht or Hb.

Conflict of interest
None declared.
References


