Platelets are central players in haemostasis and atherothrombosis. Their nucleated progenitors, the megakaryocytes (MKs), reside in the bone marrow and have unique features. During differentiation, MKs first undergo a series of endomitoses, accumulating DNA up to 64–128% in a single cell; thus, mature MKs are the largest bone marrow cells, with a characteristic hyperthrophic nucleus and oversized cytoplasm (Figure 1). Once endomitoses are completed, MKs proceed to their final, ‘suicidal’ maturation, whereby all the cytoplasm is progressively converted into pro-platelets, through pseudopod formation, elongation into the bone marrow sinusoids, and pro-platelet packaging (Figure 1). Pre-platelets are then released as large, round, anucleated MK fragments which undergo a final binary division to discoid, mature platelets. The newly formed platelets that enter the peripheral blood have peculiar morphological and functional features: they represent the largest platelets, rich in RNA [therefore also designated ‘reticulated platelets’ (RPs)] and display a maximal pro-haemostatic capacity.

Based on their systemic bioavailability, antiplatelet drugs interact with peripheral platelets as well as with MKs, pro-platelets, and pre-platelets in the bone marrow. However, because of the limited accessibility of MKs, RPs provide potential tools for indirectly probing the pharmacological effect of antiplatelet drugs on MK drug targets and the carry-over effect from central MKs to peripheral platelets, and assessing the relevance of the drug target renewal rate. Moreover, due to their higher functional reactivity, RPs might contribute to atherothrombotic complications in certain clinical settings.

The hypothesis linking larger platelets to the pathophysiology of acute coronary syndromes (ACS) was first put forward by John Martin and Colleagues in the early 1980s. Almost concomitantly, a role for cyclooxygenase (COX) acetylation in MKs was hypothesized to explain the delayed recovery (2-day lag) of platelet COX-1 after low-dose aspirin withdrawal. Without COX isozyme acetylation in MKs, ~10–12% (and a higher proportion under conditions of accelerated platelet turnover) of newly formed platelets with intact COX-1 activity would appear in the systemic circulation during the 24-h dosing interval of low-dose aspirin administration; while in fact COX-1 activity, as reflected by whole blood thromboxane (TX) B2 production, is suppressed by 99% throughout the dosing interval.

Similar considerations would apply to thienopyridines (ticlopidine, clopidogrel, and prasugrel) which, like aspirin, are characterized by a short-lived active moiety (the active metabolite) permanently inactivating the platelet drug target, i.e. P2Y12. Thus, for both aspirin and thienopyridines, interindividual variability in the duration of the antiplatelet effect might be related to variable rates of renewal of the platelet drug target (COX-1 and P2Y12, respectively) during the 24-h dosing interval. In contrast, blockade of platelet P2Y12 by reversible inhibitors (ticagrelor and cangrelor) is closely related to circulating drug levels; b.i.d. dosing of ticagrelor ensures adequate plasma concentrations to achieve persistent P2Y12 inhibition throughout the 12-h dosing interval (Figure 1).

Several studies have described an association between large platelets and/or RPs and coronary heart disease, including ACS, correlating platelet turnover to clinical manifestations of atherothrombosis. However, whether a higher fraction of large platelets is a cause or consequence of ACS (e.g. from platelet consumption on atherosclerotic plaques and consequent accelerated turnover) remains to be determined. Moreover, it is unknown whether the proportion of RPs might decline over time after an acute vascular event, in part due to effective antiplatelet therapy. Interestingly, a reduction in RPs 12 months post-myocardial infarction (MI) has been described recently, consistent with the initial reports of large platelets decreasing after MI.

The paper by Bernlochner et al. in the current issue of the journal confirms and extends previous evidence by establishing a link between RPs and platelet responsiveness to the P2Y12 inhibitors prasugrel and ticagrelor in 124 ACS patients randomized in the ISAR-REACT 5 trial. The investigators analysed the RP fraction, ADP-induced platelet aggregation as assessed by the Multiplate Analyzer (MEA), and the expression of P-selectin on ADP-stimulated RPs at two time points during maintenance drug intake (in a subgroup of 28 patients). The main findings of this study are: (i) ~18–19 h after the loading dose, residual ADP-induced platelet response to ticagrelor and prasugrel in patients with acute coronary syndrome, by I. Bernlochner et al., on page 3202.
aggregation was correlated to RPs in the prasugrel group but not in
the ticagrelor group, in spite of a higher average platelet inhibition
associated with prasugrel treatment; and (ii) on maintenance ther-
apy, RPs expressed significantly more P-selectin in response to
ADP at the end of the dosing interval (i.e. 1 h before the next intake)
as compared with an earlier determination (2 h after the last intake)
in the prasugrel group (26.3% vs. 11.9%) but not in the ticagrelor
group (13.5% vs. 9.4%). The fact that overall platelet inhibition, as
assessed by MEA, was below the threshold for high platelet reactiv-
ity in both groups confirms the limitations of arbitrary thresholds of
these functional measurements.5 A correlation between high RPs
and reduced prasugrel-induced inhibition of platelet aggregation in
patients with ST-segment elevation MI (STEMI)14 as well as a lack
of correlation between RPs and the degree of residual platelet
aggregation in non-ST segment elevation (NSTE)-ACS patients on ticagrelor were recently reported and are consistent with the present findings. Study limitations include: (i) lack of parallel measurements of plasma drug levels, when the ‘early’ and ‘late’ P-selectin expression on ADP-stimulated RPs were measured; and (ii) lack of repeated measurements to assess the stability of the platelet phenotype over a 12-month treatment period.

An inverse correlation between RPs and antplatelet responsiveness to low-dose aspirin has also been reported in diverse clinical settings such as type 2 diabetes, stable coronary heart disease, ACS, and essential thrombocythaemia. Independently of the underlying disorder, a lower platelet inhibition was consistently observed in aspirin-treated patients with the highest RPs. Therefore, the mechanism(s) linking RPs to suboptimal antplatelet response seems to be independent of the drug class and underlying disease, but rather dependent on the mechanism of drug action (irreversible vs. reversible inhibition), on the half-life of the active drug, and on the kinetics of platelet turnover. An extreme clinical paradigm of this unfavourable combination is exemplified by essential thrombocythaemia, a myeloproliferative neoplasm characterized by persistently enhanced platelet production. In essential thrombocythaemia patients treated with low-dose aspirin, RPs independently predicted poor aspirin responsiveness. A more frequent dosing regimen (100 mg b.i.d.) restored the extent and duration of platelet inhibition, while a higher once-daily dose (200 mg) was not as effective.

What are the clinical implications of the study of Bernlochner et al.? First, their findings suggest that in ACS patients treated with prasugrel the duration of P2Y12 blockade may be shorter than 24 h, at least in a variable fraction of patients with accelerated renewal of the drug target. Whether more persistent inhibition of platelet P2Y12 by the conventional b.i.d. regimen of ticagrelor confers a detectable clinical advantage over prasugrel in this setting remains to be established by the 12-month outcome of the ongoing head to head comparison performed by the ISAR-REACT 5 Investigators. Secondly, the results of this study may allow interpretation of the negative findings of the CURRENT OASIS-7 trial, in which doubling the once-daily dose of aspirin and/or clopidogrel in ACS patients did not affect their vascular outcome to any statistically significant extent. As shown in type 2 diabetes and in essential thrombocythaemia, doubling the once-daily aspirin dose does not increase the duration of its antplatelet effect, while a b.i.d. regimen prevents the time-dependent recovery of COX-1 activity during the dosing interval. Thirdly, the present results provide a rationale for a b.i.d. dosing study of prasugrel in ACS patients with high RPs. Finally, in order to develop a personalized approach to antplatelet therapy, we clearly need more extensive studies of RPs in different clinical settings, as well as sensitive and reliable mechanism-based biomarkers of antplatelet drug responsiveness. These data would be essential to inform in silico models in order to simulate the impact of individual pharmacokinetic and pharmacodynamic features of the drug in the face of variable rates of renewal of the drug target.

Conflict of interest: B.R. reports receiving consulting honoraria from Merck Sharp and Dohme Italia; C.P. reports being an unpaid member of the Scientific Advisory Board of the Aspirin Foundation and receiving an institutional grant from Bayer AG for investigator-initiated research.

References