**Identification of a distinct platelet phenotype in the elderly: ADP hypersensitivity co-exists with platelet protease-activated-receptor (PAR)-1 and PAR-4 mediated thrombin resistance**.

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**Aim:** Thrombin (via PAR-1 and PAR-4 receptors) and adenosine diphosphate [ADP] (via P2Y12 receptors) are potent endogenous platelet activators implicated in the development of cardiovascular disease (CVD). While the frequency of CVD increases with age, the benefit of antiplatelet agents diminishes in the elderly. We aimed to assess whether platelet pathways altered with ageing.

**Method:** We characterised platelet activity in community-dwelling volunteers (n=174) recruited at Concord Hospital, Sydney, in the following age groups: (i) 20-30 (young); (ii) 40-55 (middle-aged); (iii) ≥70 years (elderly). Platelet activity was assessed by whole blood aggregometry; flow cytometry (surface markers [P-selectin: alpha granule release, CD63: dense granule release, PAC-1: GPIIb/IIIa conformational activation] measured under basal conditions and after agonist stimulation [ADP, thrombin, PAR-1 agonist or PAR-4 agonist]; receptor cleavage and quantification; fluorometry; calcium flux; ELISA.

**Results:** The elderly had higher basal platelet activation than the young, evidenced by increased expression of P-selectin, CD63 and PAC-1, which correlated with increasing inflammation (interleukin-1β). The elderly demonstrated higher P2Y12 receptor density, greater ADP-induced platelet aggregation and lower stored intracellular ADP (all p<0.05). However, elderly subjects were resistant to thrombin, achieving less activation in response to thrombin (higher EC50) and to selective stimulation of both PAR-1 and PAR-4, with higher basal PAR-1 and PAR-4 receptor cleavage and less inducible PAR-1 and PAR-4 cleavage (all p<0.05). Thrombin resistance was attributable to a combination of reduced thrombin orienting receptor GPIbα, reduced secondary ADP contribution to thrombin-mediated activation and blunted calcium flux. D-Dimer, a marker of *in-situ* thrombin generation, correlated with platelet activation in the circulation, *ex-vivo* thrombin resistance and circulating inflammatory mediators (TNF-α).

**Conclusion:** Ageing is associated with a distinctive platelet phenotype of increased basal activation, ADP hyperreactivity and thrombin resistance. *In-situ* thrombin generation associated with systemic inflammation may be a novel target to prevent and treat CVD in the elderly.

