Thrombocytopenia model with minimal manipulation of blood cells allowing whole blood assessment of platelet function

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Appendix I

Gating strategy

Figure 1

Identification of platelets were based on the cell size (forward scatter) and CD42b expression on the surface (A) and subsequently in a forward scatter (height)/forward scatter (area under the curve) plot, where platelet-platelet aggregates were excluded (B). This leaves only single platelets to be investigated in the platelet function analyses. Analysis of the platelet function was performed based on 10.000 platelet events.

Figure 2

The x-axes are fluorescence intensity of the applied antibody, whereas numbers of events are displayed on the Y-axes (count). Platelet preactivation was defined as platelets expressing P-selectin with no agonist addition applying the gate from the negative control (Figure 2A). The negative control is the first top (red), which contained isotype-Allophycocyanin (APC) and ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson Bioscience, San Jose, CA, USA), whereof the latter was added to inhibit platelet activation. Gates in the negative control were set to 1-2% of platelets binding fibrinogen, 1-2% with CD63 expression, and 0.1-0.2% with p-selectin expression according to Rubak et al. (Platelets, 2015, Apr 22:1-9.).

Platelet function was defined as the expression of platelet markers (bound fibrinogen, CD63 and P-selectin) identified by fluorescence labelled antibodies on the platelet surface and quantified on a log scale. After agonist addition, the platelet function was evaluated as the median fluorescence intensity (MFI) of all platelets (green top) (Figure 2B-D).