Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Washed platelet assay, the heparin-induced platelet activation (HIPA) test

Platelets were purified from ACD-A anticoagulated whole blood obtained from healthy donors who did not take antiplatelet medications or non-steroidal anti-inflammatory drugs (NSAIDs) during the previous 10 days, by differential centrifugation. Platelet-rich plasma was washed with washing buffer, resuspended in Tyrode's buffer containing 0.35% bovine serum albumin and 0.1% glucose. Heat-inactivated (56 °C, 30 min) patient serum (20 µL) and washed platelets (75 µL) were incubated in a microtiter plate with either buffer, 0.2 aFX U/mL low-molecular-weight heparin, reviparin (Abbott, Germany; if reviparin is not available, enoxaparin can be used), 100 IU/mL unfractionated heparin, or 10 µg/mL PF4 (final concentration, Chromatec, Germany) in the presence and absence of the FcγIIa receptor- blocking antibody (IV.3, obtained by cell supernatant, cell line ATCCHB-217, Biometec GmbH). The microtiter plate was incubated (45 min, RT) on a magnetic stirrer (500 rpm) with two steel spheres (2 mm diameter). The transparency of the suspension was assessed using an indirect light source every 5 min. The test is considered positive if platelet aggregation occurs within 30 min in at least 2 of 3 (or 2 of 4) test cells in the presence of PF4 alone (or that occurs in buffer) and that is inhibited at high heparin.

Affinity purification of Serum IgG by biotin-PF4 and biotin-PF4/H coupled magnetic beads

Coupling of biotinylated PF4 (biotin-PF4) (Cat.No. 006/16; Chromatec, Greifswald, Germany) to streptavidin-conjugated paramagnetic microbeads (Dynabeads-SA) (Cat. No. 65601, Dynabeads MyOne Streptavidin T1, Invitrogen) was performed according to the manufacturer's instructions. Briefly, 250 µL Dynabeads-SA were washed four times with 2 mL PBS (pH 7.4) and resuspended in 250 µL biotin-PF4 (400 µg/mL PBS). For each sample, 250 µL biotin-PF4 and Dynabeads-SA were coincubated for 30 min at room temperature with gentle rotation and washed four times with 500 µL washing-buffer (PBS pH 7.4 supplemented with 0.1% BSA) by magnetic separation. 200 µL of serum was added after the last magnetization step, and the sample was incubated for 90 min at 37°C under gentle rotation. Beads were then washed four times in 500 µL washing-buffer. After the last magnetization step, 400 µL acidic elution-buffer (0.1 M glycine, pH 2.7) was added for 1 min. The eluate was subjected immediately to a 100k-centrifugal filter device (Amicon Ultra-2, Merck Millipore, Darmstadt, Germany) and centrifuged for 5 min, 4000xg. Samples were washed with an additional 400 µL elution-buffer, centrifuged again, and the remaining 100-130 µL supernatant immediately neutralized with 10 µL Tris-HCl-buffer (1 M, pH 9.0). The protein concentration of each sample was measured at 280 nm on a NanoDrop2000 photo spectrometer (ThermoFisher, Waltham, USA) against the respective blank (glycin-buffer or TRIS-neutralized glycin-buffer).

For affinity purification of serum IgG-elution from biotin-PF4/heparin coupled Dynabeads-SA, PF4/H complexes of 1.0 IU/mL unfractionated heparin (Heparin-Natrium 25000 IE/5mL; Ratiopharm) with 40 µg/mL biotin-PF4 were formed in 12.5 mL PBS at room temperature for 1 h. The coupling to washed Dynabeads-Streptavidin

(250 µL per sample) was performed consecutively in two steps with 2500 µL of the complex-solution (2x 1250 µL) for 30 min each and with subsequent steps performed as described above.