LPS-induced expression and release of monocyte tissue factor in patients with haemophilia

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Legends to electronic supplementary material

Online Resource 1: LPS dose titration and comparison of whole-blood monocytes to PBMCs

(**A** and **B**) Peripheral blood mononuclear cells (PBMCs) were isolated from citrate-anticoagulated whole blood and stimulated with various concentrations of lipopolysaccharide (0–10 μg/mL) for 4 h at 37°C. Expression of TF antigen on CD14-positive monocytes was subsequently analysed by two-colour flow cytometry. Results are presented as TF-specific mean fluorescence intensity (MFI, **A**) or as the proportion of TF-positive cells (**B**). A representative experiment with different scaling of the x-axis is shown. (**C**) Citrate-anticoagulated whole blood or isolated PBMCs were incubated with buffer (PBS) or 10 μg/mL LPS for 4 h at 37°C before monocyte TF antigen was analysed by flow cytometry (mean ± SD, n=10). Results are presented as TF-specific MFI (left panel) or percent TF-positive cells (right panel). P values are according to two-sided Student's *t*-test.

Online Resource 2: Analysis of monocyte TF antigen by two-colour flow cytometry

(**A**) The region R1 indicates whole-blood monocytes labelled with a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against CD14. (**B**) Gated monocytes were stained for TF antigen using a phycoerythrin (PE)-conjugated monoclonal antibody (anti-TF) in comparison to PE-conjugated control IgG. Samples were analysed immediately after blood draw (baseline) and after incubation for 4 h with buffer (PBS) or 10 μg/mL lipopolysaccharide (LPS). TF-positive monocytes were identified by a gate arbitrarily set to include 1 % of monocytes treated with control IgG. Representative histograms from a healthy male control and a patient with haemophilia A are shown. Arbitrary units (AU) of TF-specific mean fluorescence intensity (MFI) and percentages of TF-positive monocytes, obtained after subtracting values in the presence of control IgG from those in the presence of anti-TF, are indicated in red.

Online Resource 3: Monocyte TF antigen expression in patients and controls and correlation of TF parameters in LPS-treated patient samples

(**A**) TF antigen on whole-blood monocytes was analysed by two-colour flow cytometry both at baseline and after incubation for 4 h at 37°C with buffer (PBS) or lipopolysaccharide (LPS). Results are presented as percent TF-positive monocytes. (**B**) The proportion of TF-positive monocytes was plotted against TF-specific MFI of monocytes in LPS-treated patient samples. Correlation coefficient (*r*) and P value are according to the method of Pearson. (**C**) MV-associated TF PCA was plotted against TF-positive monocytes in LPS-treated patient samples. Correlation coefficient (*r*) and P value are according to the method of Spearman. The value for MV TF PCA is missing for one patient.

Online Resource 4: Correlations between hs-CRP/IL-6 and LPS-induced monocyte TF antigen in the patient cohort

Baseline serum levels of hs-CRP (**A**) and IL-6 (**B**) were plotted against monocyte TF antigen,

expressed as the proportion of TF-positive cells. Values were obtained from LPS-treated patient samples. Correlation coefficients (*r*) and P values are according to the method of Spearman.

Online Resource 5: Correlation between whole-blood leukocytes and LPS-induced TF parameters in the patient cohort

Baseline whole-blood leukocytes were plotted against monocyte TF antigen, expressed as TF-specific MFI (**A**) or percent TF-positive cells (**B**), and MV-associated TF PCA (**C**). Values were obtained from LPS-treated patient samples. Correlation coefficients (*r*) and P values are according to the method of Pearson (**A** and **B**) or Spearman (**C**). The values for MV TF PCA is missing for one patient.

Online Resource 6: Effect of the HBV/HCV infection status on whole-blood leukocytes, LPS-induced monocyte TF antigen and age in the patient cohort

Baseline whole-blood leukocytes (**A**), LPS-induced monocyte TF antigen, expressed as the proportion of TF-positive cells (**B**), and age (**C**) are shown for healthy male controls (n=23) and patients with (n=16) or without positive HBV/HCV test results (n=27) at study inclusion. P values are according to ANOVA and Tukey´s post-hoc test.

Online Resource 7: Correlation of the OJS with whole-blood leukocytes and effect of clinically significant arthropathy on age in the patient cohort

(**A**) Baseline whole-blood leukocytes were plotted against the orthopaedic joint score (OJS). Correlation coefficient (*r*) and P value are according to the method of Spearman. (**B**) Age is shown for healthy male controls (n=23) and patients with (n=22) or without clinically significant arthropathy (n=21) at study inclusion. P values are according to ANOVA and Tukey´s post-hoc test.

Online Resource 8: Exploratory subgroup analyses of LPS-induced TF parameters and inflammatory markers

Data are presented as mean ± standard deviation or as median and inter-quartile range. *P values (for the comparison of respective patient subgroups with controls) are according to two-sided Student's *t*-test or Mann-Whitney U test. OJS ≤ 4 indicates absence of clinically significant arthropathy. Values for MV TF PCA are missing for one patient and two controls. Abbreviations are as follows (in alphabetical order): AU, arbitrary units; HA, haemophilia A; HB, haemophilia B; HBV, hepatitis B virus; HCV, hepatitis C virus; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LPS, lipopolysaccharide; MFI, mean fluorescence intensity; MV, microvesicle; n.a., not applicable; n.d., not determined; OJS, orthopaedic joint score; PCA, procoagulant activity; TF, tissue factor; w/o, without.