The staphylococcal toxin Panton-Valentine Leukocidin induces neutrophil extracellular trap (NET) formation that leads to platelet activation.

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**Objective:** Expression of the toxin Panton-Valentine Leukocidin (PVL) by Staphylococcus aureus has been linked to thrombosis in association with osteomyelitis especially in young immunocompetent patients. We therefore tested the effect of PVL on human platelets and neutrophils.

**Methods:** Human neutrophils were gently isolated from whole blood. The effect of recombinant PVL on platelet activation in the presence or absence of autologous neutrophils was measured by flow cytometry. PVL induced lysis of neutrophils was assessed by propidium iodide. Release of neutrophil myeloperoxidase and defensins was detected by ELISA. NET formation was analyzed by a fluorogenic assay using Syto13 binding to nucleic acids, a sandwich ELISA against histone-DNA complexes and by microscopy. The influence of known inhibitors of alpha defensin and of FDP-lysine carrying proteins induced platelet activation was studied.

**Results:** The toxin PVL strongly induced platelet activation, but only in the presence of human neutrophils. Labelled PVL subunit S (LukS) bound to the neutrophil surface, but not to platelets. Complete PVL induced neutrophil lysis and NET formation as well as the release of alpha defensins and myeloperoxidase. Neutrophil NETs were decorated with alpha defensin 1-3, and stained for FDP-lysine, a marker for acrolein adduct formation that induces platelet activation. PVL-induced platelet activation in the presence of neutrophils was inhibited by known defensin inhibitors as well as by resveratrol and glutathione (GSH). PVL actions were blocked by anti-PVL-antibodies in the plasma of several adult blood donors. GSH also inhibited PVL-induced lysis of human neutrophils and the release of defensins and myeloperoxidase.

**Conclusion:** Our data provide one possible explanation how thrombosis and osteomyelitis are connected in PVL-S. aureus infections and why especially young osteomyelitis patients with a presumably low antibody titer against PVL suffer more from this complication than adults. As the mechanism described might be a more general one, other bacterial toxins are under further investigation.
Prothrombin fragment F1+2 in pregnancy is associated with thrombophilic risk factors and a history of venous thromboembolism (VTE)

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Objective: Since the positive predictive value of factor V Leiden (FVL) and other genetic risk determinants for a pregnancy-associated VTE is low, additional indicators of hypercoagulability are needed to identify women at risk of VTE during pregnancy. An increased level of prothrombin fragment 1+2 is a potential indicator of hypercoagulation in normal pregnancy. We hypothesized that women with previous VTE and FVL or prothrombin G20210A mutation are at a higher hypercoagulable state during pregnancy than women without prior thrombotic complications and without genetic thrombophilic risk factors.

Methods: In a prospective study, we determined prothrombin fragment F1+2 over pregnancy (818 measurements) among 131 women with previous VTE, 109 women with previous fetal loss (1 late or 3 early fetal losses), 51 women with previous severe preeclampsia, and 93 healthy pregnant women. Evaluation of thrombophilia included factor V Leiden and prothrombin G20210A mutation. The prothrombin fragment F1+2 levels were statistically analyzed over the course of pregnancy using a multivariate mixed model.

Results: Among women with a previous history of VTE, prothrombin fragment F1+2 values were significantly higher during the course of pregnancy than among pregnant women without VTE (p<0.0001) (p=0.63 for preeclampsia vs. no preeclampsia, p=0.40 for fetal loss vs. no fetal loss). The results were adjusted for the physiological increase of prothrombin fragment F1+2 over pregnancy (p<0.0001) and independent of heparin prophylaxis (prothrombin fragment F1+2 reduced, p=0.004). In addition, FVL (p=0.004) and prothrombin G20210A mutation (p=0.011) were independently associated with increased levels of prothrombin fragment F1+2.

Conclusion: Increased prothrombin fragment F1+2 in pregnancy are associated with thrombophilic risk factors (FVL and prothrombin G20210A mutation) and a history of VTE. Determination of indicators of hypercoagulation such as prothrombin fragment F1+2 can represent a supplementary approach to identifying women at risk of VTE during pregnancy, independent of known and unknown risk determinants of VTE.