Infections, coagulation and thrombosis
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The aim of this thesis was to further explore the hypothesis that a virus-induced procoagulant change is one of the mechanisms behind the association between these viral infections and venous thromboembolism and cardiovascular disease. We investigated the changes in coagulation during and immediately after viral respiratory tract infections in an animal model and in the general population, and cytomegalovirus infection in kidney transplant patients. Furthermore, we investigated the association between viral respiratory tract infections and pulmonary embolism and cardiovascular disease. Lastly, we describe a bacterial disease and its association with venous thrombosis.

In Chapter 2 we reviewed the literature on viral infections and their effect on the coagulation cascade and the resulting (venous) vascular complications. We discussed both non-hemorrhagic and hemorrhagic viruses. Non-hemorrhagic viruses mainly cause thrombotic complications, but bleeding complications have been described, although these are uncommon. Hemorrhagic viruses cause both thrombotic and bleeding complications, the latter frequently preceded by disseminated intravascular coagulation. These viruses have a high morbidity and mortality. Viral infections can have an impact on the initiation, propagation and inhibitory phases of the coagulation cascade. Furthermore, thrombocytopenia due to various mechanisms may occur, including autoantibodies directed against platelets. Research on coagulation disorders due to viral infections has been performed on different levels of the coagulation cascade; uniformity in investigations could not be observed. Therefore, similarities in mechanisms between various viruses have not been ascertained. Further research is needed in a uniform way to reveal common pathways, before general preventive and treatment strategies can be developed.

Chapter 3 concerns a prospective study in a cohort of human subjects of 55 years of age and older, selected from a General Practitioners office in Overveen (n=53). We studied the course of changes in markers of coagulation in blood samples during and two weeks after a naturally occurring respiratory tract infection compared with baseline blood samples. The results of this study showed that during and immediately after such an infection several of these markers were significantly elevated. We showed endothelial cell perturbation, as indicated by increased levels of von Willebrand factor (VWF), and activation of fibrinolysis, as indicated by elevated levels of plasmin-alpha2-antiplasmin (PAP) complexes. Plasminogen activator inhibitor-1 (PAI-1) was not elevated. Thrombin generation was unchanged, as shown by similar levels of prothrombin fragments 1+2 during the acute phase compared with baseline. In the convalescent phase (two weeks after infection) PAP levels were still elevated. These findings indicate that respiratory tract infections are possibly a risk factor for developing thrombosis due to a procoagulant state.

Chapter 4 is similar to the study described in chapter 3, but concerns a new cohort of subjects (n=371) from the same source population and we tested for more coagulation markers. Due to a mild influenza season, the number of subjects with a respiratory tract infection was low (n=15). This study also showed that natural occurring respiratory tract infections in human subjects result in endothelial cell perturbation (elevated levels of VWF) and an increased fibrinolytic state (elevated levels of PAP and D-dimer) with the potential for increased coagulation (elevated levels of endogenous thrombin potential (ETP) and increased resistance to activated protein C (APC-sr)).
Because most of these markers are associated with an increased risk for ischaemic heart disease and venous thromboembolism, we suggest that the induced haemostatic changes may form a link between acute respiratory tract infections and acute atherothrombotic disease.

Chapter 5 describes the effect of reconstituted high density lipoprotein (rHDL) on markers of coagulation in influenza-infected C57BL/6 mice. Activation of coagulation due to influenza infection was significantly attenuated by administration of rHDL, as shown by a decrease in influenza-induced thrombin generation (decreased thrombin-antithrombin complex levels). In addition, an improvement of fibrinolysis (combination of increased D-dimer levels and decreased PAI-1 levels) was observed after administration of rHDL to influenza-infected mice. Whether this effect is due to HDL-induced reduction of inflammation or due to a direct effect of HDL on coagulation could not be established.

In Chapter 6 the association between influenza infection and an increased risk of pulmonary embolism was studied. Therefore, we conducted a nested case control study in a large cohort of patients with a clinical suspicion of having pulmonary embolism. Influenza detection in serum samples was performed. We compared case patients, in whom pulmonary embolism was proven (n = 102), to controls, in whom pulmonary embolism was excluded (n = 395). Influenza was more frequently found in the control group than in the case group (4.3% versus 1.0%, respectively, odds ratio 0.22; 95% CI: 0.03–1.72). Therefore, we could not establish an association between influenza and an increased risk of pulmonary embolism. Furthermore, we compared symptoms of influenza-like illness in both patient groups 2 weeks prior to inclusion in the study, using the influenza-like illness (ILI) score, which is based on a questionnaire. We found that the ILI-score was non-specific in this clinical setting. It is conceivable that influenza infections do occur more often in the control patients, since respiratory tract infections can cause a clinical picture that may be very similar to that of a pulmonary embolism, and influenza infections are one of the more common alternative diagnoses among patients suspected of having a pulmonary embolism.

Chapter 7 describes a study on the association between viral respiratory tract infections and acute coronary syndrome (ACS), which includes myocardial infarction and unstable angina. We investigated the presence of viruses that cause respiratory tract infection in blood samples by means of antibodies, and in throat swabs by means of PCR, which were collected from patients admitted to the Coronary Care Unit (CCU) with an ACS (n=41). Furthermore, we investigated the presence of preceding clinical symptoms of a respiratory tract infection. As a control group, patients from the outpatient clinic of the department of Internal Medicine without evident cardiovascular disease were selected, matched by age and sex. We controlled for calendar time, and thereby for confounding by seasonal fluctuations in the circulation of respiratory viruses, by including control subjects within 7 days from inclusion of the matching case patient. The findings of this prospective pilot study did not confirm an association between recent respiratory tract infection and a higher risk of ACS. Our results suggest approximately equal risks of ACS for patients with and without a laboratory proven recent respiratory viral infection, but the confidence intervals were wide. In addition, there was no relationship between clinical signs of a respiratory tract infection and confirmative laboratory investigations.

In Chapter 8 we describe a prospective study in a cohort of 70 renal transplant recipients to determine whether CMV infection induces a prothrombotic state in vivo. We compared
haemostatic proteins and activation markers between patients with and without active CMV (n=29). We did not find statistically significant differences in any of the haemostatic markers. In addition, we tested for changes in haemostatic markers comparing samples of subjects when positive for CMV to samples of the same individual when CMV negative. During CMV infection levels of plasmin-α2-antiplasmin (PAP) complexes were significantly increased, and von Willebrand factor (VWF) levels were only somewhat higher, while no changes in any of the other haemostatic markers were observed. We showed a significant association between CMV load and levels of D-dimer. The results of this study suggest increased fibrinolysis and possibly also endothelial cell perturbation, as evidenced by increased levels of PAP and VWF, respectively. It is not clear whether the net effect of these alterations leads to less or more thrombus formation. Whether these changes are responsible for the observed association between CMV infection and vascular disease remains subject of further study.

In Chapter 9 we presented two cases of patients suffering from Lemierre’s syndrome and a review about this syndrome. Lemierre’s syndrome is a potentially fatal disease that usually presents with oropharyngeal infection, followed by sepsis, thrombosis or suppurative thrombophlebitis of the internal jugular vein and septic emboli predominantly to lungs and joints. Lemierre’s syndrome is a rare disease with an estimated incidence of approximately one per million persons per year, and despite appropriate therapy, mortality can be as high as 4–18%. Because it is a rare disease, it may therefore be often misdiagnosed at early presentation. Most cases are caused by the Gram-negative, anaerobic bacteria Fusobacterium necrophorum. Although this chapter concerns a bacterial pathogen unlike the previous chapters, it is a clinical example of the relationship between infections and venous thrombosis and its complications.