This thesis describes work performed at the intersection of coagulation and liver disease. Chapter 1 relates the work in this thesis to previously performed studies and external developments. A short introduction in haemophilia, coagulation disorders in liver disease and current views on the induction of coagulation is given.

Chapter 2 consists of a review of literature on hepatitis C (HCV) in haemophilia patients. After some background information, the prevalence of infection is discussed. Subsequently, studies on the clinical course of chronic hepatitis C are reviewed. The second half of the paper discusses data on treatment of chronic hepatitis C infection in haemophilia patients.
We concluded that the burden of liver disease in haemophilia patients will probably increase in the next 10 to 20 years. Treatment of chronic hepatitis C is available but at the time of writing only effective in about 40% of patients. There is no consensus on who should and who should not be treated. If treatment is opted for, combination therapy of interferon and ribavirin must be first choice. The optimal dose of interferon and length of treatment is not yet known.

Chapters 3 and 4 describe studies on lymphocyte subsets in haemophilia patients. Abnormal lymphocyte subsets in human immunodeficiency virus (HIV) negative haemophilia patients have been described previously in detail, but their cause remains unclear. Treatment with impure concentrates has been implicated but it has also been hypothesized that, in parallel with HIV infection, HCV infection can be the cause of these abnormalities.

In chapter 3, we indirectly compared two different groups of HCV positive, HIV negative haemophilia patients. We described seven severe haemophilia B patients, before and after substitution was changed from prothrombin complex concentrate to monoclonally purified concentrate. Data were compared with healthy controls and previous findings in haemophilia A patients.
At baseline, haemophilia B patients did not differ from controls. After two years, T helper cells showed an increase, while a rise in B cells approached statistical significance. In contrast, haemophilia A patients showed increased numbers of activated non-B lymphocytes and lowered numbers of B cells at baseline as compared to controls. After two years activated non-B lymphocytes decreased, as did the CD4/CD8 ratio, due to increasing numbers of CD8 positive cells. Our data suggested minor inhibition of the immune system in haemophilia B patients, which

recovered after changing therapy to a monoclonally purified product. These findings contrasted with the excessive immune stimulation in haemophilia A. We concluded that this difference does not support a causative role for hepatitis C.

In chapter 4, we compared lymphocyte subsets in 20 HIV negative, HCV positive haemophilia A patients and in 14 non-haemophiliacs with chronic hepatitis C to those in 18 healthy controls. In haemophilia patients, higher numbers of T lymphocytes and activated CD8 positive cells, lower numbers of B lymphocytes and a normal CD4/CD8 ratio were seen. These differences were either not demonstrated in hepatitis C controls or were less pronounced and in the opposite direction. From this study, we concluded that haemophilia A patients show abnormalities in lymphocyte subsets that could not be attributed to chronic hepatitis C.
In both studies, we speculated whether the observed differences might be due to the administered concentrates.

Chapter 5 is concerned with the natural history of hepatitis C. Most available data have limited usefulness for counselling patients and planning treatment, as they come from highly selected patient groups. This limitation can be overcome in patients with haemophilia or other congenital coagulation disorders who are followed for their primary disorder irrespective of liver disease. They form a homogeneous, well-defined group with a known mode and duration of infection. We studied a group of 45 HCV-RNA positive, HIV negative patients, mainly haemophiliacs, from our centre. None had received specific therapy for chronic hepatitis C.
A prospective protocol of medical history, physical examination, laboratory tests and abdominal ultrasonography was performed in all patients. Patients were classified according to results of ultrasonography.
We found that two patients had experienced an episode of variceal bleeding, all others were asymptomatic. None had ascites. HCV-RNA titers were >500.000 copies/ml in 23 patients, genotype was 1 in 31 patients. Forty (89%) had elevated transaminases, liver synthesis function was diminished in 7 (16%), platelet count in 8 (18%). Ultrasonography was normal in 26 (58%) patients while 12 (27%) had isolated splenomegaly and 7 (16%) had liver nodularity compatible with cirrhosis. Univariate analysis disclosed higher transaminases and γGT, older age at acquisition of infection and older present age as risk factors for more advanced disease. Of these, only older present age was an independent predictor in multivariate analysis. We concluded that a median 19

years after infection, 58% of patients had no other signs of liver disease than raised transaminases and 16% had cirrhosis on ultrasonography. Only 2/45 patients had symptomatic disease. Older present age was in this group the main risk factor for advanced disease.

In chapter 6, we described a multicentre, randomised, double-blind, placebo-controlled trial of induction dosed interferon-alpha combined with ribavirin in patients with congenital coagulation disorders and chronic hepatitis C.

As reviewed in chapter 2, many patients with congenital coagulation disorders have chronic hepatitis C. In chapter 5, we showed that a significant proportion of an unselected group of this patients has signs of liver disease.

Standard therapy for chronic hepatitis C is a combination of interferon-alpha and ribavirin. Pilot studies have reported higher succes rates when interferon-alpha therapy is started with a high dose induction scheme, but the safety and feasibility of very high doses of interferon-alpha is doubted and no double-blind comparisons of different doses are available. We wanted to assess if starting combination therapy for chronic hepatitis C with an induction dose of interferon-alpha was feasible and would increase response.

Sixty-six patients with congenital coagulation disorders and chronic hepatitis C from six Dutch haemophilia centres were included. They received either 5 MU interferon twice daily (induction therapy) plus ribavirin for four weeks or 5 MU interferon every second day (standard therapy) plus ribavirin for 4 weeks, in both groups followed by interferon 6 MU every second day for another 48 and ribavirin for another 22 weeks. We defined sustained response as negative HCV-RNA PCR at 6 months after the end of one-year therapy. Adverse events were monitored.

Sustained response was 57% in induction therapy versus 41% in standard therapy (p 0.215). Dose adjustments were needed in 53% of patients (62% in induction therapy, 41% in standard therapy, p 0.093). Treatment was prematurely discontinued in 8% of patients.

We concluded that the overall rate of sustained response in this trial was high and that, although no statistically significant difference between induction and standard therapy could be demonstrated, all comparisons favoured induction therapy. Induction therapy was safe and feasible, even in hemophilia patients, although intensive monitoring and frequent dose adjustments were necessary. Double-blind comparisons of interferon-alpha doses and the use of placebo injections were realistic.

In chapter 7, we reported three cases of clinical experience with recombinant factor VIIa (rFVIIa), one in a patient with acquired thrombocytopathia and two in patients with vascular pathology.

Chapters 8, 9 and 10 describe different aspects of a pilot trial of rFVIIa in orthotopic liver transplantation (OLT). From a haematological viewpoint, OLT is characterised by extensive vascular damage in a coagulation-compromised patient. Control of bleeding has been a major challenge from the start of OLT. It was shown that large transfusion requirements, i.e. excessive blood loss, during OLT are correlated with increased morbidity and mortality.
Recombinant FVIIa has improved haemostasis in a variety of conditions, but had never been studied in liver transplantation. We performed a single centre, open label, pilot study in six adult patients undergoing OLT for cirrhosis Child-Pugh B or C. Eighty μg rFVIIa/kg was administered at the start of the operation. Packed red blood cells (RBC), fresh frozen plasma (FFP) and platelet concentrates (PC) were administered according to predefined criteria.

Chapter 8 focuses on safety and efficacy of rFVIIa in OLT. Perioperative transfusion requirements in six study patients were compared to matched controls, adverse events were monitored.
Transfusion requirements [median (range)] were lower in the study group than in matched controls: 1.5 (0-5) versus 7 (2-18) units allogeneic RBC, 0 (0-2) versus 3.5 (0-23) units autologous RBC, total amount of RBC 3 (0-5) versus 9 (4-40) units. Transfused FFP was 1 (0-7) versus 8 (2-35) units. Blood loss was 3.5 L (1.4-5.3) versus 9.8 L (3.7-35.0). All differences were statistically significant. One study patient developed an hepatic artery thrombosis at day 1 postoperatively.
We concluded that a single dose of 80 μg/kg rFVIIa significantly reduced transfusion requirements during OLT and that further study was needed to establish the optimally effective and safe dose of rFVIIa in this setting

In chapter 9, we assessed the effect of rFVIIa on coagulation and fibrinolysis during OLT.
In the six patients who had received a single dose of 80 μg/kg rFVIIa and in ten untreated controls, coagulation factors, parameters of thrombin generation and parameters of fibrinolysis were measured during and after OLT.
We found that coagulation factor concentrations did not differ between patients and controls. Thrombin generation did not rise after the administration of rFVIIa, but did sharply increase after reperfusion in patients as compared to controls. No difference in fibrinolysis was seen between patients and controls. No evidence of diffuse intravascular coagulation was seen.
We concluded that the use of rFVIIa in OLT enhanced thrombin generation in a localized and time-limited matter, without causing systemic coagulation.

Chapter 10 examines whether the haemostatic effects of rFVIIa in patients with cirrhosis, in a stable situation or during the OLT pilot study, might be explained in part by enhanced downregulation of fibrinolysis by the thrombin activatable fibrinolysis inhibitor, TAFI. Addition of therapeutical or supratherapeutical doses of rFVIIa to plasma of 12 patients with stable cirrhosis did not result in a prolongation of clot lysis time, whereas clotting times were significantly reduced. Also, clot lysis assays of plasma samples taken during and after OLT, in study patients and controls, did not show any effect of rFVIIa on plasma fibrinolytic potential. We concluded that this study showed no evidence for an antifibrinolytic effect of rFVIIa in cirrhotic patients.

We started this project with a few clear questions, as explicated in Chapter 1. Regarding the immune abnormalities in HIV negative haemophilia patients, we have demonstrated that hepatitis C infection is not the cause. Whether the use of impure concentrates is, remains unclear. During the span of this thesis no clinical implications have emerged, but follow-up will go on. We must remain watchful for new complications of therapy with coagulation factor concentrates.

We showed that hepatitis C has caused significant liver damage in our patient group and we expect more clinical liver disease in the future. Sadly, two deaths due to liver disease have occurred in our centre in the last years. The safety of concentrates has increased greatly after the catastrophe of HIV infection, and new hepatitis C infections have not occurred. However, for previously infected patients, chronic hepatitis C remains a real and ongoing threat. They have a right to the best chances of cure from this disease.

Therapy for chronic hepatitis C has greatly improved with the introduction of ribavirin. We showed that with high-dosed combination therapy 50% sustained response is possible. We did collect data to answer the question whether the immune abnormalities are related to success of treatment, but these are not yet analysed.

High costs should not be an impediment to an effective and safe treatment of haemophilia. A significant part of our knowledge of trombosis and haemostasis is derived from haemophilia and other congenital coagulation disorders. This has benefitted the treatment of other patient groups. The use of recombinant factor VIIa is recent example of this.