Summary

This thesis deals with some clinical and laboratory aspects of two haematological disorders, namely paroxysmal nocturnal haemoglobinuria (PNH) and primary myelofibrosis (PMF). In both disoders precursors of the red and white blood cells as well as the thrombocytes are involved. This could point to a clonal genesis of the two disorders.

In chapter I the purpose of the study is explained. An answer was sought to the questions concerning the occurrence and significance of the immunologic dysfunction in PMF and PNH; and to what degree this is caused by lymphocytes belonging to the affected clone in PNH. It is possible that lymphocytes would show signs of dysfunction as well as have characteristics found in the other cells of the affected clone. Therefore immunologic capacity was tested in PMF and PNH, and lymphocytes of PNH patients were studied for their increased sensitivity to complement i.e. the 'PNH-defect'. Furthermore this chapter describes various pathophysiological aspects of bone marrow fibrosis, especially the vascular aspects.

In chapter II a review of the literature is presented concerning pathogenesis, coexistence with other haematological disorders, treatment and complications of PNH.

Chapter III reports the results of various immunological studies in five patients with PNH. A disturbed cell-mediated immunity was observed, while the immunoglobulin levels and the α-Helix Pomatia Haemocyanin (HPH)-antibody response were normal. It was concluded that humoral immunity was normal, although the B-cell number was depressed. Furthermore it appeared that the presence of inulin, an activator of the alternative pathway of the complement system, resulted in a decreased in-vitro lymphocyte proliferation response, possibly induced by a dysfunction in a subset of lymphocytes with the 'PNH-defect'.

However, monocytes have an important role in the in-vitro lymphocyte stimulation tests, therefore it can not be excluded that these may have contributed to the disturbed function.

Based on these data, further analysis of the T-cells of the PNH patients was done. This was performed by typing with monoclonal antibodies, measurement of concanavalin-A (con-A) induced suppressor cell activity

and studies with Indium^{111}-oxine labelled lymphocytes, as is described in chapter IV.

Analysis of T-cell subsets with monoclonal antibodies demonstrated a variable decrease of the percentage of OKT_3 and OKM_1 positive cells, while the ratio OKT_4/OKT_8 was significantly depressed. Although the phenotyping with monoclonal antibodies points to an increased suppressor cell activity, this was not supported by a functional test, namely the con-A induced suppressor cell activity.

The studies with the Indium^{111}-oxine labelled lymphocytes did not indicate an abnormal sensitivity when the lymphocytes were exposed to normal or acidified serum.

These data suggest that an imbalance of T-cell subsets exist in PNH, probably as an inherent feature of the underlying disease, for there is no convincing evidence concerning the expression of the 'PNH-defect' in the lymphocytes of the PNH patients.

In chapter V two patients with PNH, who developed severe vascular complications, are described. One patient developed extensive thrombosis of the intra-abdominal veins, while the second had an aneurysm of the abdominal aorta. The pathogenesis of thrombosis in PNH and its therapeutical consequences are discussed.

Chapter VI reports the results of different immunological studies in ten patients with PMF, namely the in-vitro lymphocyte proliferation tests with mitogens, DNCB skin reactions, and the α-HPH antibody response.
In addition the results of the complement factors (C3, C3a and C4) and various circulating immune-complex (CIC) assays as the indirect granulocyte phagocytosis test, the Clq-binding and the polyethylene glycol precipitation test are reported.

Cell-mediated immunity was disturbed, as manifested by depressed in-vitro lymphocyte stimulation with phytohaemagglutinin and con-A and disturbed DNCB skin reactions, especially in the patients with long standing PMF. Diminished Ig-class specific antibody titres were detected against haemocyanin, especially the IgA-class, indicating a disturbed humoral immunity. Levels of CIC were also increased in the patients with a long-standing PMF, especially with the Clq-binding test. These data suggest that the immunologic dysfunctions in PMF are a feature of the progressive advanced disease.

Chapter VII describes the results of the quantitation of procollagen type-III (PC-III) in PMF and PNH. This peptide is released during the conversion of procollagen to collagen by fibroblasts.
Significantly increased levels were observed in PMF, especially in those

patients with an active disease, while the levels in the PNH patients were normal.

During a follow-up of several months, two patients with PMF were treated with acetylsalicylic acid. This resulted in a decrease of the β-thromboglobulin level, although normalization did not occur. At the same time the PC-III level declined.

It was demonstrated by means of Sephadex gel filtration that the antiserum of PC-III not only reacts with PC-III but also with other peptides.

These data demonstrate that the radioimmunoassay cannot be used for the quantitative determination of PC-III. Nevertheless it gives some insight in the process of bone marrow fibrosis.

Whether anti-thrombotic agents could have a limited role in the prevention of bone marrow fibrosis remains to be evaluated.