During the recent years, increasing knowledge has been obtained regarding patients with thrombocytopenia with a normal number of megakaryocytes in the bone marrow. Especially platelet kinetic studies have been helpful to categorise the different subgroups related to decreased production by bone marrow dysfunction or thrombocytopenia due to increased peripheral destruction.

Chapter III describes the use of two $^{111}$In ligands, $^{111}$In-oxinate and $^{111}$In-tropolonate. First, a series of in vitro investigations has been performed in order to measure the labelling efficiency and the viability of autologous platelets labelled with $^{111}$In-tropolonate compared to $^{111}$In-oxinate. Using $^{111}$In tropolonate, it was possible to label autologous platelets in a plasma environment, thus improving the labelling efficiency to 95% as compared to 60% with $^{111}$In oxinate. In physiological saline no difference was observed: both ligands showed labelling efficiencies over 90%. $^{111}$In ligands enable autologous platelet labelling even at a platelet count below 25 x $10^9$/L. Loss in platelet viability could not be demonstrated since there was no obvious difference in aggregation with adenosine diphosphate (ADP) before and after platelet labelling, neither with $^{111}$In-tropolonate nor with $^{111}$In-oxinate. Based on the favourable results with $^{111}$In-tropolonate, a complete set of in vivo investigations, including mean platelet life and organ measurements were performed using tropolonate only. It was demonstrated that the spleen rapidly took up autologous platelets labelled with $^{111}$In-tropolonate. In normals the uptake lies within a sharply defined range, and remains constant for five to seven days. Similar results were obtained from the liver uptake. In thrombocytopenic patients however, MPL and PPR vary from normal to greatly reduced. These results indicate that the labelling procedure with $^{111}$In-tropolonate can be used adequately to perform platelet kinetic studies.

In chapter IV the value of platelet kinetics is described in a ‘homogeneous’ group of 141 ITP patients. It was to be expected that the majority would present with decreased MPL and normal to increased compensatory platelet production. Indeed, bone marrow analysis demonstrated a normal to increased number of megakaryocytes in the majority of cases. However, no correlation was observed between the morphological findings and PPR determined with the platelet kinetic studies. Based on these results, patients with ITP can be divided into two sub-groups. The first group showed the conventional picture of ITP (short MPL, normal to increased PPR and increased destruction in the spleen). The second patient group (40%) demonstrated ineffective thrombopoiesis reflected by longer MPL, and a decreased PPR.

In view of the heterogeneity of the patients groups, the impact of therapy needed to be evaluated (Chapter V). ITP patients were studied before and during prednisone therapy ($n=17$) and before and after splenectomy ($n=24$). In the prednisolone group, it was shown that patients responsive to therapy demonstrated especially an improvement in PPR, with minor effects on MPL. All patients with a normal platelet count after prednisolone preserve a significant shortened MPL.

In contrast, patients responsive to splenectomy and normalisation of peripheral platelet count demonstrated especially normalisation of the MPL and PPR in contrast to non-responsive patients. These facts suggest that although prednisone increases PPR, complete response seems only possible if, at the same time MPL is prolonged. These findings suggest that both prednisolone and splenectomy reduce the amount of platelet associated autoantibodies. However, complete remission occurred only in splenectomized patients.

76
In chapter VI a patient selection was made based on reduced PPR in the platelet kinetics, in order to analyse whether PPR was due to reduced platelet production in the bone marrow, reflected by increased TPO levels, or due to increased medullary destruction translated into an elevated GC-Index.

TPO plasma levels were not statistically different from normal controls; neither could a significant difference between ITP patients with a normal to increased PPR versus patients with a decreased PPR be demonstrated. In contrast, GC-index in ITP patients with reduced PPR was significantly higher than in patients with normal to increased PPR. This discrepancy between a decreased number of platelets released into the circulation and an increased release of GP-Ib complex suggests shedding of the receptor complex from megakaryocytes and/or platelets destructed in the bone marrow. However it is not likely that this is associated with a reduced mass of TPO bearing cells in view of the normal TPO levels. The finding that no significant correlation existed between GC-index and MPL further supports the hypothesis of increased medullary destruction. The results indicate that in a sub-group of ITP patients thrombocytopenia may be caused by increased megakaryocyte- and platelet destruction in the bone marrow.

Finally, chapter VII describes another application of autologous labelled platelets. Platelet kinetics were performed in eight patients with paroxysmal nocturnal haemoglobinuria (PNH) in order to define the cause of thrombocytopenia.

The majority of PNH patients had reduced PPR, indicating ineffective thrombopoiesis since bone marrow examination demonstrated hyper- or normocellular bone marrow. It was remarkable that virtually all PNH patients showed increased accumulation of platelets in the abdominal blood vessels, not associated with clinical signs of thrombosis. PNH patients have an increased tendency to thrombosis especially in abdominal veins. This might be partly due to increased tendency of platelets to adhere to the endothelial cell layer. The findings indicate that platelet kinetics have no value for the diagnostic process of thrombosis in PNH. Alternatives have to be used such as Ultrasound imaging and MRI.

**FUTURE PERSPECTIVES FOR THROMBOCYTOPENIA**

This thesis demonstrates that contrary to the ASH ITP practice guidelines (Blood 1996; 88: 3-40), ITP cannot be considered a homogeneous disorder, but that it appears to be divided into different subgroups. Platelet kinetic studies have revealed that two sub-groups of patients can be recognised whereby a significant difference is observed with regard to the release of platelets in the circulation i.e. the PPR. Platelet kinetic studies and plasma GC and TPO levels indicate that the reduced PPR in a subgroup of patients is related to an increased intramedullar destruction of platelets and megakaryocytes. These findings may require alternative approaches for classification, diagnosis and treatment.

The results described in chapter VI indicate that the GC-I is an important tool to distinguish these subgroups of ITP patients and it may be of use for further classification of ITP patients.

In addition, alternative methods may be developed to label platelets. In 1993, trace labelling of platelets with biotin in whole blood has been described. The in vivo behaviour and the life span of biotinylated platelets is similar to platelets labelled with radionuclides, suggesting that this method might be an appropriate substitute in humans also. The label adheres to all platelet cohorts and they can be monitored by flow cytometric techniques, so
risk effects are minimal. Because of these advantages, this technique may be an ideal alternative to radionuclidic methods. Possible toxic and immunological effects will have to be monitored closely before this method can be introduced for widespread human use.

Sofar the determination of platelet associated immunoglobulins does not have important diagnostic or prognostic value. However, the results in the subgroups of ITP patients suggest that antibodies are not only directed to platelets but also to megakaryocytes. Moreover different subtypes of autoantibodies may be present since no difference in MPL was observed in patients with a reduced versus normal or increased PPR. Further research will have to focus on the identification of the platelet autoantibodies.

Preliminary results indicate that increased apoptosis of megakaryocytes is observed in patients presenting with ITP. Whether this can be related to patients not responding to prednisone and splenectomy has to be evaluated. Demonstration of apoptosis of megakaryocytes in patients with ITP would evidently open up the possibility of new therapeutic options especially for non-responsive patients. Agents that affect this process of apoptosis might be of great relevance including agents like the haematopoietic growth factor TPO that might prove of eminent value for the treatment of patients with ITP.