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CLINICAL OBSERVATIONS IN THROMBOCYTOPENIA

Klinische observaties bij trombocytopenie

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Promotor	Prof.dr. B. Löwenberg
Overige leden:	Prof.dr. A. Brand
	Prof.dr. J. Lindemans
	Prof.dr. P.Sonneveld

CONTENTS

CHAPTER 1

INTRODUCTION

Thrombocytopenia.
Platelets have a central role in maintaining normal hemostasis and vessel wall repair. Decreased platelet numbers (thrombocytopenia) increase the risk of bleeding. Thrombocytopenia is a frequently encountered clinical problem. The causes of thrombocytopenia can roughly be divided into 4 main categories, failure of platelet production, increased destruction or consumption of platelets, sequestration of platelets (distributional thrombocytopenia) and massive blood loss (dilutional thrombocytopenia).
Increased platelet destruction occurring mainly extravascular, is seen in a number of situations of auto- and alloimmunisation. In contrast, the intravascular consumption of platelets is particularly seen in such conditions as thrombotic thrombocytopenic purpura (TTP), the physical destruction during cardiopulmonary bypass, or the destruction within vascular malformations as in giant cavernous hemangiomata and large aortic aneurysms.

Severe thrombocytopenia may be life-threatening and requires urgent therapeutic intervention. This may involve for instance, discontinuation of the causative drug in case of drug-induced thrombocytopenia or the supplement of platelets in cases of extreme platelet loss. However, when an immunological mechanism is active, these measures are generally insufficient or not (immediately) effective and treatment may be problematic.

We investigated clinical and laboratory aspects in 3 different categories of thrombocytopenia in which an immune mechanism is active, i.e. thrombocytopenia caused by autoantibodies (thrombocytopenia related to the anticoagulant drug heparin and autoimmune thrombocytopenic purpura), thrombocytopenia and platelet alloimmunization and a platelet consumption type of thrombocytopenia (thrombotic thrombocytopenic purpura).

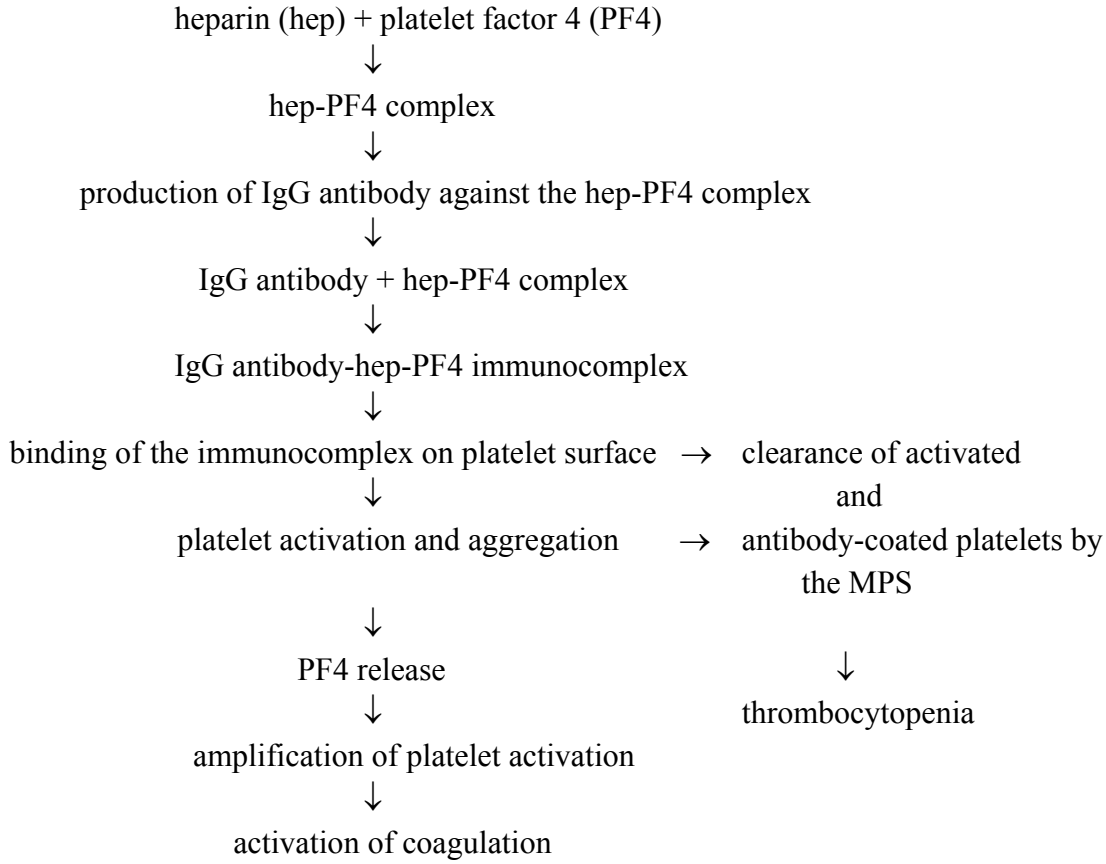
Drug-related thrombocytopenia.
Many drugs have been identified as a cause of thrombocytopenia. In the development of drug-related thrombocytopenia immunological and non-immunological mechanisms can be distinguished. Definite proof of the diagnosis may be difficult because multiple clinical variables can be involved and there are no absolute diagnostic tests.
We studied a peculiar, immunological type of thrombocytopenia caused by heparin in which a thrombotic tendency predominates an expected bleeding risk caused by a low platelet count. Thrombocytopenia is a relatively frequently encountered complication of heparin treatment.
Despite the recent development of new anticoagulant agents, unfractionated heparin (UFH) is still commonly used for the prevention and treatment of thrombotic disorders, especially in patients undergoing bypass surgery and in those admitted to the intensive care unit.

The pathogenesis of heparin-induced thrombocytopenia can be immunological or non-immunological. The non-immunological interactions between heparin and platelets are thought to be related to its proaggregatory effect depending on the charge density and chain length of heparin (1). The non-immunological type of heparin-induced thrombocytopenia is seen more frequently, i.e. in approximately 25% of patients treated with UFH. The thrombocytopenia is generally mild with nadir platelet counts rarely declining below 80-100 x

10^9/l and with an early onset, apparent already within 2-4 days after initiation of UFH (2). The thrombocytopenia resolves spontaneously within a few days even during continuation of heparin and apparently has no clinical consequences.
We studied the more serious, immunological form of heparin-induced thrombocytopenia. A decrease of the platelet count typically begins 5 to 10 days after the start of UFH. This interval is the time required to initiate an immune response. In acute HITT a more rapid fall of the platelet count may occur, apparent at a median of 10.5 hours after the start of UFH. Delayed-onset thrombocytopenia will occur several days after cessation of UFH. As in typical-onset HITT, acute- and delayed-onset HITT are believed to result from the interaction between heparin and circulating platelet factor4 antibodies formed following recent heparin exposure (<3 months) (3). Upon heparin discontinuation, platelet counts generally recover within 5 days but it may take several weeks in patients with high titer antibodies.
The immunological type of heparin-induced thrombocytopenia may be associated with thrombosis in up to 50% of the patients. The thrombosis generally occurs a few days before thrombocytopenia becomes manifest but may also appear after heparin cessation, usually within the first week (4, 5, 6). A variety of thrombotic complications are seen in HITT. Venous thrombosis complicates HITT more often than arterial thrombosis. Venous thrombosis includes upper and lower limb veins, pulmonary embolism and rarely the adrenal veins. Arterial thrombosis most often involves the lower limb arteries. Cerebral and coronary artery thrombosis are seen less often. The risk of fatal thrombosis is about 5% (7).
For practical purposes we use the term “HITT”, heparin-induced thrombocytopenia and/or thrombosis in our study.

The mechanism of HITT is that of an immune response (see figure). The small peptide platelet factor 4 (PF4) is a platelet-specific chemokine that is stored within the alpha granules of platelets. Under normal conditions trace levels of PF4 are found in the plasma. PF4 binds heparin with high affinity.
Heparin is a glycosaminoglycan consisting of polymers with variable chain length and variable levels of sulphation. Heparin binding to PF4 occurs more efficiently with the larger and higher sulfated heparins than with low-molecular-weight heparins (9). The binding of heparin gives rise to a complex in which PF4 becomes antigenic. Following a conformational change of the platelet factor 4-heparin (PF4/hep) complex, a cryptantigen is exposed to which the formed antibodies bind. The antibodies are usually of IgG_1 subclass (10).
Subsequently, PF4/hep/antibody complexes bind to platelets via their Fc receptors (FcγRII). The binding of these antigen/antibody complexes leads to platelet activation and additional PF4 release. Amplification of this process then results in enhanced platelet activation and aggregation. Subsequent clearance of activated and antibody-coated platelets by the mononuleair-phagocytic system may cause thrombocytopenia.

Figure. Pathophysiology of HITT.*



*Adapted from Franchini et al (8).

During the process of platelet activation, platelet procoagulant material is generated which results in thrombin formation (11). Thrombin plays a central role in HITT. Thrombin generation is enhanced by several other processes including generation of platelet microparticles, alteration of endothelial cells and production of tissue factor by endothelial cells and monocytes (12, 13, 14). The resulting prothrombotic state may hold on for some days or weeks even after discontinuation of heparin therapy (5, 6).

The diagnosis of immunological HITT is made on clinical criteria but should be confirmed by laboratory tests. These tests are to exclude HITT-like conditions such as diffuse intravascular coagulation.
Heparin-dependent antibodies (HITT antibodies) are detected either by functional (platelet activation) assays or by antigen assays. HITT antibodies cause strong platelet activation resulting in platelet release reaction and platelet aggregation which are used as an endpoint in tests for the detection of the antibodies (15, 16). Antigen assays, such as a solid phase immunoassay, measure the binding of antibodies to immobilized PF4/hep complexes (17). Immunoassays are technically easier to perform and have greater sensitivity for HITT

antibody detection than platelet activation assays. On the other hand, functional tests, such as platelet activation tests, perform better as regards the assay of HITT antibodies that are clinically significant (18, 19). Classically, platelet aggregation assays have been used to detect platelet activating HITT antibodies. Aggregation of platelets is detected in a standard platelet aggregometer after platelets are activated in the presence of the patient's serum and heparin. However, these tests are cumbersome. In 1991 a faster and sensitive test based on direct visualization of heparin-induced platelet activation (HIPA) in microtiter wells has been described by Greinacher et al (16).

The early reports on the incidence of HITT are inconclusive and mainly concern surgical patients (20). We conducted a prospective cohort study on the incidence and timing of HITT in medical patients. The majority suffered from heart disease and the others presented with neurological disease. As, at the time of designing our study, there were no established criteria for the diagnosis HITT, we first defined HITT. Since it appeared from the literature that in many patients treated with UFH, heparin-dependent antibodies are demonstrable without ever developing clinical manifestations, we also evaluated two different laboratory tests for the detection of HITT antibodies, i.e. a platelet activation assay (HIPA) and an antigen test (PF4/hep immunoassay) (16, 17). *The results of these studies are described in* ***chapter 2****.*

Thrombocytopenia and platelet alloimmunization.
Transfusion of blood platelets is an indispensable part of the supportive care of patients with congenital and acquired thrombocytopenia. It is particularly important in patients with acute leukemia and other malignancies treated with chemotherapy. Prophylactic platelet transfusions are given during the period of severe thrombocytopenia following the cytotoxic treatment. Refractoriness to random donor platelet transfusion has been reported to occur in the majority of patients in the pre-filtration era (21). Once a patient becomes refractory, effective prophylaxis of hemorrhage by platelet transfusion is at risk.

Refractoriness to random donor platelet transfusions can be mediated through immunological and non-immunological factors. Examples of non-immunological causes which may compromise post-transfusion platelet survival, are septicaemic states and administration of antibiotic or anti-fungal drugs, diffuse intravascular coagulation and splenomegaly.

The major immunological cause of refractoriness to platelet transfusions is alloimmunization of the recipient by alloantigens present on donor platelets. Clinically relevant alloantigens carried by platelets are: human leukocyte antigen (HLA) class I antigens, platelet specific antigens (human platelet antigens, HPA) and antigens of the ABH blood group system. HLA alloimmunization represents the main cause of platelet transfusion refractoriness and is seen in 50-90% after transfusion of non-leukocyte-depleted blood components and in 0% to 28% of cases after leukocyte-depleted blood components (22, 23, 24).

HLA antibody formation requires both HLA class I as well as HLA class II bearing, antigen presenting cells (primary HLA immunization). As platelets lack HLA class II antigens, they

are unable to provoke a primary immune response on their own. Studies in animals and humans demonstrated that allogeneic leukocytes are required to induce HLA antibody formation (25). Already by the end of the 80s it was shown that systemic leukoreduction of blood products reduces the probability of platelet alloimmunization and prevents platelet transfusion refractoriness (22, 26, 27). Later on it was shown that rigorous leukocyte depletion to less than 5 x 10^6 per unit transfused, resulted in further reduction of refractoriness against random donor platelet transfusions to an average of only 3% of patients (22, 24, 28). But, although primary alloimmunization has been eliminated to a considerable extent after the introduction of universal leukoreduction of blood products, patients with a transfusion history of non-leukodepleted blood products or with previous pregnancies remain at risk of developing HLA antibodies in 30-60% of the cases (secondary immunization) despite the administration of leukodepleted platelet transfusions (21, 28, 29).
HPA alloimmunization causes neonatal alloimmune thrombocytopenia, post-transfusion thrombocytopenic purpura and may also lead to refractoriness to random donor platelet transfusions. The frequency of HPA antibodies is not precisely known but is estimated to be approximately 5-15%, often in combination with HLA antibodies (30). Isolated HPA antibodies only rarely (<5%) cause refractoriness to random donor platelet transfusions (23, 31, 32). ABO incompatibility between donor and recipient also has been reported to compromise platelet transfusion efficiency but it is of moderate practical consequence and may be prevented by the administration of ABO-matched/compatible platelet transfusion products (33, 34).

For the clinician it is useful to be informed about the presence of platelet alloantibodies. This will allow to discriminate between immunological and non-immunological etiologies of platelet transfusion refractoriness. Moreover, HLA antibody testing of the recipient may provide a predictor of HLA-matched platelet transfusion outcome. In the past a variety of assays for HLA- and platelet specific allo-antibodies have been developed. The current practice in our department in case of refractoriness to random donor platelet transfusions is to screen for the presence and nature of anti-platelet antibodies with the platelet immunofluorescence test (PIFT). This assay is combined with a more specific test for the detection of HLA-antibodies, either a lymphocyte immunofluorescence test (LIFT) or a monoclonal antibody-specific immobilization of platelet antigens assay (MAIPA) (35, 36). The PIFT assays for an increased amount of immunoglobulins bound to platelets. The LIFT measures HLA antibodies. By MAIPA, HLA- and platelet specific antibodies can be detected, depending on the monoclonal antibody used.
Data in the literature concerning the predictive value of these or other tests for HLA-matched platelet transfusion outcome in populations not being exposed to non-leukocyte depleted transfusions, are scarce (37). In ***chapter 3*** *we report a study on the predictive value of HLA-antibody testing for the outcome of the first HLA-matched platelet transfusion in thrombocytopenic patients refractory to random donor platelet transfusions who always had received leukocyte depleted blood products in case of transfusion.*

Thrombocytopenia caused by platelet autoimmunization.

Primary immune thrombocytopenic purpura (ITP) is an autoimmune disorder with an estimated incidence of 1.6 to 6.6 cases per 100.000 per year (38). The disease is characterized by autoantibody-mediated platelet destruction. The platelet-antibody complexes are recognized by Fcγ-receptors expressed on macrophages in the mononucleair phagocytic system, in particular in the spleen. This results in premature clearance of platelets from the circulation, a reduced platelet lifespan and thrombocytopenia.

In the 1950s Harrington was the first to postulate the immune pathogenesis of the disease. He infused plasma from ITP patients into normal volunteers which resulted in a rapid and severe fall in platelet counts with a return to normal in 4-6 days (39). Autoreactive B cells secreting anti-platelet antibodies are considered to be the immunological intermediators in ITP but the initial cause of the autoimmune response in ITP is unknown. More recently, pathogenic T cell responses have also been demonstrated in chronic ITP, indicating the presence of activated platelet-specific autoreactive T cells that drive the autoreactive B cells (40, 41). In addition, several other mechanisms have been suggested to be active in ITP. Autologous platelet survival studies unexpectedly showed normal or only slightly increased platelet survival in up to two thirds of ITP cases. This argues for an impairment of thrombopoiesis in ITP (42, 43, 44). Other arguments for impaired thrombo- and megakaryopoiesis are the various morphologic (ultrastructural) abnormalities in megakaryocytes that are seen seen in ITP, the in vitro inhibitory effect of ITP plasma samples on megakaryopoiesis and the induction of apoptosis in cultured megakaryocytes by ITP plasma (45, 46, 47).

The different pathophysiological mechanisms that may operate in ITP suggest that ITP is a heterogeneous disease. This may explain the variations in diagnostic test results and clinical symptomatology and the observed differences in response to treatment. Since there are no specific diagnostic tests nor any clinical findings specific of the disease, ITP remains a diagnosis by exclusion.

The diagnosis is established when, no other cause for thrombocytopenia is apparent, the number of megakaryocytes in the bone marrow is normal or increased and the spleen is not enlarged. The presence of platelet autoantibodies as demonstrated by a positive direct platelet immunofluorescence test adds further support to the diagnosis as does a shortened lifespan of Indium-111-labeled autologous platelets.

Treatment recommendations in ITP are hampered by the fact that well-defined prospective studies comparing different treatment regimens are scarce. Moreover, no parameters predicting treatment outcome are known. Based on the presumed different causative mechanisms in ITP, therapy can target in three different ways, a) inhibition of macrophage Fcγ-receptor function, b) inhibition of antibody production and c) stimulation of platelet production (48).

Immunosuppressive therapy has been the corner stone of treatment in ITP. Corticosteroids, which classically are used in the treatment of ITP, probably inhibit Fcγ-receptor function, reduce antibody production and may increase platelet production. First-line treatment with corticosteroids has reported success rates of about 15-60%. Only a minority attain a durable remission. Splenectomy is the treatment of choice for those in whom corticosteroids are

ineffective. Splenectomy will remove a primary site of antibody synthesis as well as a site of platelet clearance through the removal of macrophage Fc-receptors. After splenectomy, durable remissions are reached in about 70% of the cases (38). But splenectomy is an invasive procedure and has the (long-term) risk of infectious complications. Moreover, the therapeutic effect of splenectomy is unpredictable.
Over the years a variety of immunosuppressive regimens have been introduced for the treatment of splenectomy refractory cases. Examples of these immunosuppressive agents are azathioprine, cyclophosphamide, cyclosporin-A (CyA) and mycophenolate mofetil which all inhibit both B and T cell function (49). However, the comparative value of each of these agents has remained unclear until now. Moreover, none of these drugs have been evaluated in minimally-treated, non-splenectomized patients.
Based on the assumption of a T cell-mediated mechanism for aberrant B-cell activity in ITP, we tried to improve outcome of disease by applying intensified immunosuppression with the combination of corticosteroids and CyA. In ***chapter 4*** *the results of this combined immunosuppressive regimen in corticosteroid refractory patients with ITP, with or without splenectomy, are reported.*

The role of thrombopoietin in autoimmune thrombocytopenia.
The hematopoietic growth factor thrombopoietin (TPO) has been implicated in the pathophysiology of ITP. It has also been proposed as a therapeutic agent in ITP. TPO has an important role in the production of megakaryocytes and platelets. TPO acts at several maturation stages of megakaryocytopoiesis via the thrombopoietin receptor Mpl which is expressed on the cell surface of megakaryocytes and platelets.
The TPO-gene is located on chromosome 3q27-28. The amino-terminal of the TPO molecule bears 21% sequence homology with erythropoietin (50). This domain binds with high affinity to its receptor, Mpl (51). The carboxy-terminal of TPO is the unique part of the molecule, which has no homology to any known protein. The main production sites of TPO are the liver (hepatocytes) and the kidneys (proximal tubulus) whereas small amounts are produced in the stromal cells of the bone marrow and the brain.
The uptake of TPO by megakaryocytes and circulating platelets is a major mechanism in the regulation of the TPO levels. Other mechanisms regulating TPO levels include upregulation of TPO production at the transcriptional level by bone marrow stroma cells, release of TPO by activated platelets and proteolytic cleavage of TPO by thrombin (52, 53, 54). The numbers of circulating thrombocytes and bone marrow megakaryocytes are therefore determinants of the amount of free TPO in the circulation.

TPO can be detected by a sensitive and specific ELISA. The minimal detectable amount of TPO is 1 AU/ml (arbitrary unit). Because TPO is released from platelets during blood clotting, serum TPO levels are 3.5 times as high as plasma TPO levels(55).
Measurement of serum or plasma TPO levels may be useful in the differential diagnosis of thrombocytopenic states. Patients with aplastic anemia who exhibit a decreased or absent megakaryocyte population show elevated TPO levels as compared to normal individuals. In contrast, in patients with platelet destruction as a consequence of auto- or alloantibodies, no

increase in TPO is seen (table 1) (56, 57). These findings are consistent with the model of TPO levels being regulated by Mpl-mediated degradation. In case of increased platelet destruction in the spleen, as is the case in ITP, TPO is destroyed together with platelets at an accelerated rate in the mononucleair phagocytic system. Since the spleen thereby acts as a kind of TPO sink, the compensation for the decreased platelet count by an increased platelet production in the bone marrow seems to be insufficient (58, 59). This has provided a circumstantial argument for the treatment of ITP patients with TPO. Currently clinical studies with thrombopoietin preparations are in progress (60).

Table 1. Thrombopoietin levels in thrombocytopenia

disease state	thrombopoietin
chemotherapy-induced aplasia	high
aplastic anemia	very high
myelodysplastic syndromes	heterogeneous/ generally increased
immune thrombocytopenia	normal or slightly increased

We studied two aspects of thrombopoiesis in 35 patients with ITP, i.e. serum TPO levels before and after treatment and platelet turnover rates as measured with Indium-III-labelled autologous platelets in a subset of these patients. The results of these studies are described in ***chapter 5****.*

Thrombocytopenia caused by platelet consumption due to an underlying immune disturbance.

Thrombotic thrombocytopenic purpura (TTP) is an example of a platelet consumption type of thrombocytopenia in which platelets are consumed by thrombi formed in the microcirculation under the conditions of high shear-stress. TTP is a rare disease with an estimated incidence of 3.7 per million in the United States (61). It is classically characterized by thrombocytopenia, microangiopathic hemolytic anemia, neurological symptoms, fever and kidney dysfunction. All these features are rarely present at the same time. The cause of the disease has for long time been elusive. More recently, the discovery of a specific von Willebrand factor (vWF)-cleaving protease has provided new insights into the pathophysiology of TTP. The vWF seems to play a key role in this disease. VWF is synthesized in megakaryocytes where it is stored in α-granules and in vascular endothelial cells where it is stored in Weibel-Palade (W-P) bodies. The vWF in the W-P bodies is rich in ultra-large von Willebrand Factor (ULVWF) forms which bind efficiently to glycoprotein Ibα platelet membrane receptors. Upon secretion, ULVWF are rapidly cleaved in smaller sized multimers which are much less adhesive to platelets. Failure to degrade ULVWF multimers, by a specific vWF-cleaving protease, results in the persistent circulation of ULVWF leading to platelet aggregation and this elicits the clinical syndrome of TTP.

The protease has been characterized as a member of the ADAMTS (a disintegrin and metallo-proteinase activity) family of metalloproteases (62, 63). Initial reports considered

ADAMTS13 deficiency to be specific for TTP. However, we and others have demonstrated decreased ADAMTS13 activity in patients with disseminated intravascular coagulation (64, 65). Subsequently, moderately or severely decreased ADAMTS13 activity has been demonstrated in other disease states such as in liver disease, uremia and acute inflammatory disorders. Decreased ADAMTS13 activity has also been noted in newborns, pregnancy and even in healthy controls (66, 67). Meanwhile it has become evident that a severe deficiency of ADAMTS13 activity (<5% of normal) is a specific feature of acquired idiopathic TTP (68, 69).
TTP patients represent a clinically heterogeneous group. Rare congenital forms, adult acquired forms, including relapsing cases, and secondary forms of TTP can be distinguished.
Congenital deficiency of ADAMTS13 is caused by gene mutations. Acquired idiopathic TTP is thought to be caused by autoantibody formation against ADAMTS13 (70).

Treatment.
Since the introduction of plasma therapy in the sixties, the mortality of patients with acquired idiopathic TTP has declined from >90% to < 30% but the treatment of patients who are refractory and those with relapsing disease has remained problematic.
On an empirical basis, splenectomy has been advocated for many years as a treatment for intractable cases. Because of the scanty and incomplete data in the literature, we assessed the value of splenectomy in patients with refractory or relapsing TTP in a larger patient group with extended follow-up. The outcome of this evaluation is described in ***chapter 6****.*

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CHAPTER 2

HEPARIN-INDUCED THROMBOCYTOPENIA AND THROMBOSIS: A PROSPECTIVE ANALYSIS OF THE INCIDENCE IN PATIENTS WITH HEART AND CEREBRO-VASCULAR DISEASES

M.C.Kappers-Klunne M.D.[1], D.M.S.Boon M.D.[1], W.C.J.Hop[2] Ph.D., J.J.Michiels M.D.[1], J.Stibbe M.D.[1], C.van der Zwaan[3], P.J.Koudstaal M.D.[4], H.H.D.M. van Vliet Ph.D.[1]

[1]) Department of Hematology
[2]) Department of Epidemiology and Biostatistics
[3]) Department of Cardiology
[4]) Department of Neurology of the University Hospital Rotterdam

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SUMMARY

Heparin-induced thrombocytopenia and/or thrombosis (HITT) are serious complications of heparin treatment. The incidence, as previously reported, varies widely and, in consequence, is not precisely known. Moreover, most reports only concern clinically defined heparin-induced thrombocytopenia. Therefore, we performed a prospective study of the incidence of serologically confirmed HITT.

All patients admitted to the departments of cardiology and neurology of our institution with an indication for treatment with therapeutic-dose intravenous unfractionated heparin were enrolled in the study. The patients were examined daily for the occurrence of thromboembolic complications. Regular platelets and tests for the presence of heparin-dependent antibodies were carried out using two different tests: a quantitative platelet factor 4/heparin (PF4/hep) Elisa and a functional test, the heparin-induced platelet activation assay (HIPA). HITT was defined as a rapidly occurring (within 5 days) decrease of the platelet count from normal value of >120 x 10^9/l to <60 x 10^9/l or to <100 x 10^9/l if there was a rapid fall of >50% of starting value or >30% with concomitant acute thrombosis.

The observed incidence of HITT was 1/358 patients (0.3%, 95% confidence limits 0.01-1.5%). However, Elisa PF4/hep specific IgG antibodes were demonstrated in nine (2.5%) and IgM antibodies in seven (2.0%) of 358 patients. Thirty of 358 patients (8.4%) had platelet activating antibodies in the HIPA.

We conclude that the incidence of serologically confirmed HITT in this study is very low (0.3%) in patients with cardiac and neurologic diseases treated with intravenous unfractionated heparin. The frequency of heparin-dependent antibodies without concomitant occurrence of thrombocytopenia is much higher.

INTRODUCTION.

Heparin-induced thrombocytopenia and/or thrombosis (HITT) are well recognized and serious complications of heparin treatment. Generally, a rapidly falling platelet count is the first manifestation of HITT, which may be followed by the occurrence of thromboembolic phenomena in a small proportion of cases. Initial reports on the incidence of HITT, mainly concerning heparin-induced thrombocytopenia, are inconclusive with percentages varying from 0-30%. Adequately defined, prospective studies indicating the true incidence of HITT are very scarce. Analysis of 18 prospective studies only requiring a reproducible platelet count of <100 x 10^9/l, indicated an incidence of 2.9% for intravenous bovine and 1.1% for intravenous porcine heparin. In the absence of prospective data regarding the occurrence of thromboembolic complications related to heparin treatment, its frequency could only be roughly estimated to be < 1% (Schmitt and Adelman, 1993).

There are no generally accepted criteria for the diagnosis of HITT. A rapidly falling platelet count, the occurrence of a new thrombotic event during heparin treatment or unexplained heparin resistance, are indications of HITT. Restoration of the platelet count after heparin withdrawal further strengthens the diagnosis. Specific laboratory tests might support clinical suspicion.

In the past, several functional tests have been described of which the most widely used are platelet aggregation methods and a platelet ^{14}C-serotonin release assay (SRA) (Sheridan et al, 1986). Although considered the 'gold standard', the SRA is laborious and requires radioactive markers. Since platelet aggregation is technically simple it is most widely used to diagnose HITT (Chong et al, 1993). A modified, more sensitive form, the heparin induced platelet activation assay (HIPA), has recently been described (Greinacher et al, 1991). Very recently, a platelet factor 4/heparin enzyme-linked immunosorbent assay (PF4/hep Elisa) was introduced, which has the advantage of not being dependent on carefully selected fresh platelets as required for platelet aggregation testing. Persistent uncertainty about the precise incidence of HITT, and the availability of two new rapid, sensitive and specific tests, prompted us to carry out a well-defined prospective study in patients with heart and cerebrocascular ischemic disease, in order to gain better insight into the incidence of HITT and to evaluate the usefulness of PF4/heparin Elisa and HIPA for the diagnosis HITT (Amiral et al, 1992).

PATIENTS AND METHODS.

Patients.

All patients admitted from January through December 1994, to the departments of cardiology and neurology of our hospital, in whom treatment with an intravenously administered, therapeutic dose (>20.000 units/24 h) of porcine-derived unfractionated heparin (UFH) was initiated, participated in the study. Patients already receiving heparin treatment on admission were excluded.

At study entry the following data were obtained: department, sex, age, admission diagnosis, past history (particularly for previous heparin exposure), heparin indication, concomitant medication (especially the use of platelet aggregation inhibitors) and physical abnormalities.

Patients were monitored for 15 d from the initiation of UFH or until discharge if this took place earlier. Daily physical examination was performed with special emphasis on thromboembolic complications. Platelets were counted every other day, starting just before initiation of UFH (day 0). Plasma samples were obtained from citrated blood taken on day 0, on days 5 and 15 from initiation of UFH or at earlier discharge (day 15/discharge sample) and stored (-80°C) for later investigation.

Specific laboratory tests.

Day 15/discharge plasma samples from all patients were tested in HIPA assay and PF4/hep Elisa for the detection of IgG and IgM antibodies. If any positive result was obtained, day 0 and day 5 (if present) samples were then tested.

The HIPA test was performed according to Greinacher et al (1991). A test result was judged positive if it showed platelet activation with platelets of two or more donors. PF4/hep Elisa was performed as described by Amiral et al (1992). The cut-off value of a positive test for the PF4/hep Elisa was set at the mean ± 3SD of 60 healthy individuals. Plasma of a proven HITT patient (see definition of 'definite' HITT below) was used as a positive control in the test.

Definitions.

A progressive fall in platelet count of >50% or >30% with concomitant occurrence of a thromboembolic event was 'suspicious' for HITT. Definite HITT was defined by: normal platelet count (>120 x 10^9/l) before the start of therapy with UFH, rapidly progressive decrease of platelet count (within 5 d) to <60 x 10^9/l or to <100 x 10^9/l if there was a rapid fall of >50% of the starting value, or a rapidly decreasing platelet count of >30% with concomitant acute thrombosis, exclusion of other causes of thrombocytopenia, and a positive HIPA test and/or PF4/hep Elisa, and resolution of the thrombocytopenia after cessation of UFH.

The study was performed according to hospital-approved Ethics Review Committee recommendations.

Statistics.

Cumulative percentages of patients having a positive test outcome along time were determined using life-table methods and comparison of groups was done with the logrank test (Peto et al, 1976). In these analyses the day of sero-conversion was taken to be the day number mid-interval between the last negative day number and the first positive one. P=0.05 (two-sided) was considered the limit of significance.

RESULTS.

Patients.

Three hundred and seventy-two patients were included in the study, of whom 358 were evaluable. Reasons for exclusion were absence of a day 15/discharge plasma sample in 13 cases and planning of heart transplantation in one case. In none of the excluded patients was HITT suspected or diagnosed. 244 (68%) of the evaluable patients were male; mean patient age was 61.3 years, range 21-91; 325 (91%) patients had been admitted to the department of cardiology and 33 (9%) patients to neurology.

Table I. Indications for heparin treatment

Indication	patients (%)
Unstable angina	46
Acute myocardial infarction	21
Congestive heart failure	9
Stroke	7
Arrythmia	5
Miscellaneous	12

The mean follow-up duration was 9.1 days, only slightly longer than the average period of heparin treatment because of usual discharge on the day of heparin cessation. Seven patients died during the admission period; no deaths could be attributed to HITT. Indications for heparin

therapy are outlined in table I. The mean duration of heparin treatment for all patients was 8.3 ± 4.4 (SD) d. 240 patients (67%) had been previously exposed to heparin with a mean interval from prior heparin treatment episode of 52 months. Aspirin (100 mg daily) was taken concomitantly by 186 (52%) patients.

Only one patient fulfilled the definition of HITT. This 45 year-old female with end-stage renal failure, had been treated with continuous peritoneal dialysis and recently with intermittent hemodialysis. Immediately prior to each hemodialysis procedure, 2500 U UFH were administered as i.v. bolus injection, followed by continuous infusion of 2000 U/h for 4-6 h. 5 d after the eighth hemodialysis session, acute myocardial infarction occurred and heparin was started (day 0). 6 d later the platelet count dropped to 64 x 10^9/l from an initial count of 181 x 10^9/l . HITT was strongly suspected and heparin was ceased immediately. The diagnosis HITT was confirmed by the demonstration of IgG anti-PF4/hep antibodies. The HIPA test was, however, negative. The following day (day 7) she developed thrombosis of the right femoral vein at the insertion site of an intravascular catheter. Thrombosis was confirmed by ultrasound examination. Danaparoid treatment was instituted. The platelet count returned to normal on day 9, with disappearance of clinical signs of thrombosis. Hemodialysis was replaced by peritoneal dialysis. One patient was 'suspicious' for HITT (see definitions), because of a >50% decrease in the platelet count. None of the patients except the one mentioned above suffered a thrombo-embolic event during heparin treatment.

Table II. Thrombocyte counts and heparin-dependent antibody in relation to previous heparin exposure in 358 patients treated with heparin.

Platelet count	No. of patients				
	ELISA IgG[1]	HIPA[2]	previous heparin		
			yes	no	unknown
Nadir < 60x10^9/l (or) decline > 50%					
- definite HITT	++	-	1	0	0
- suspicious HITT	-	-	1	0	0
Nadir < 120 x 10^9/l, decline > 30%	-	-	1	0	0
	++++	-	1	0	0
Nadir > 120 x 10^9/l, decline > 30%	-	-	6[3]	1[3]	0
Nadir > 120 x10^9/l, no decline	-	-	204	95	14
	+	-	2	2	0
	++	+++	1	0	0
	++++	++	0	1	0
	++++	+++	1	0	0
	-	++	16	3	0
	-	+++	3	0	0
	-	++++	3	2	0

[1]- : negative; +,++, +++, ++++: mean optical density 3-5, 5-7, 7-9, ≥ 9 x SD, respectively.
[2]- : negative; ++, +++, ++++: positive test results with platelets of 2, 3 or 4 donors, respectively.
[3]- : restoration of platelet count to above pre-treatment level after withdrawal of heparin for 1 patient.

Laboratory examinations.

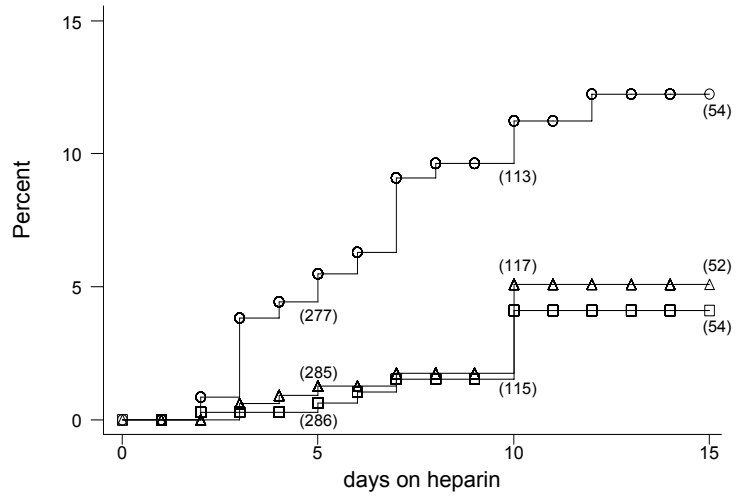
Platelet counts.

Nadir platelet counts are shown in table II. In two patients platelet counts fell by >50% of the starting value. In one of them HITT was diagnosed. The other patient was regarded 'suspicious' for HITT since laboratory tests for heparin-dependent antibodies were negative. In the other patients with declining platelet counts no specific cause was identified, nor was this a reason for discontinuation of heparin treatment.

Heparin-dependent antibodies.

HIPA and PF4/hep Elisa test results are shown in table II. The PF4/hep Elisa was found positive for IgG antibodies in 9/358 patients (2.5%) and for IgM antibodies in seven patients (2.0%, not shown). Cumulative percentages of patients with IgG and IgM antibodies on day 15 being 5% and 4% respectively (fig.1). Six patients with IgG antibodies were previously exposed to heparin. Of the seven patients with IgM antibodies, three were preexposed. Of the nine patients with a positive IgG-PF4/hep Elisa, only three tested positive by HIPA assay. There was no overlap between positive HIPA test results and the presence of IgM-PF4/hep Elisa antibodies.

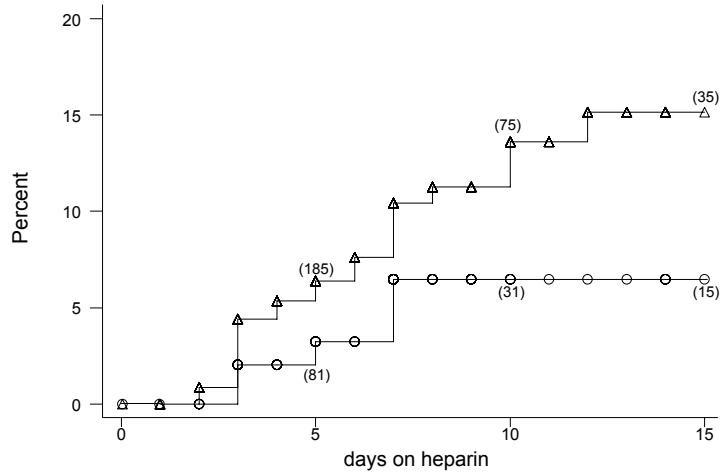
Figure 1. Cumulative (actuarial) percentage of patients with a positive test
(circles: HIPA assay; triangles: IgG; squares: IgM) according to number of days of heparin treatment.
Numbers along curves indicate numbers of patients at risk.



The HIPA test was positive in 30 patients (8.4%), 24 of whom had been previously exposed to heparin and 15 had received aspirin concomitant with heparin treatment. The cumulative percentage (at day 15) of patients with a positive HIPAA was 15% in those (n=240) who were previously exposed to heparin versus 6% for those (n=104) who were not pre-exposed (fig 2). This difference, however, is not significant (p=0.17). Data about previous heparin exposure was missing in 14 patients. In the majority (67%) the HIPA test was positive with only two of the four donor platelet suspensions tested.

Plasma samples were negative in both HIPA assay and PF4/hep Elisa in all patients tested at day 0.

Fig. 2. Cumulative percentage of patients with a positive HIPAA according to previous heparin exposure (circles: no; triangles: yes).



DISCUSSION.

We conducted a prospective study in a cohort of 358 consecutive patients treated with therapeutic-dose intravenous UFH. The majority were admitted with cardiac disease and only 9% (33 patients) suffered neurological diseases. As no difference in outcome was found between both groups, the results were pooled.

The observed incidence of HITT of 0.3% (1/358 patients; 95% confidence limits: 0.01%-1.5%) is lower than previously reported in comparable patient groups (Warkentin et al, 1991, Schmitt and Adelman, 1993). Until recently, there were no adequately designed prospective studies indicating the incidence of serologically confirmed HITT. Very recently Warkentin et al (1995) reported the results of a double-blind randomized, controlled clinical trial of prophylactic dose unfractionated porcine mucosal heparin versus low-molecular-weight heparin after elective hip surgery. Heparin-induced thrombocytopenia, defined by a decrease of the platelet count to < 150 x 10^9/l and a positive SRA test for heparin-dependent IgG antibodies after 5 d of treatment with UFH, was diagnosed in 9/332 (2.7%) patients compared to 0.3% in the current study, which is significantly lower (p= 0.009). This low incidence of HITT we found is all the more remarkable because the reported incidence for therapeutic dose intravenous heparin has always been higher than that for prophylactic, low dose, heparin.

Short-term heparin exposure may have a negative influence on the occurrence of HITT. Although the mean period of heparin treatment was 8.3 days, assumed to be long enough to develop HITT, about 20% of our patients were treated for < 5 d. However, 68% of them had been previously exposed to heparin, which, in itself, predisposes for the development of HITT. We can only speculate on the influence of the high percentage of aspirin usage (>50%) in our patient group on the occurrence of HITT, particularly in those patients with platelet activating antibodies, since reports about the efficacy of antiplatelet drugs in preventing heparin-dependent antibody-mediated platelet aggregation are scarce and conflicting (Kappa et al, 1987; Laster et al, 1989; Chong et al, 1993a).

Elisa PF4/hep specific IgG antibodies were demonstrated in 9/358 patients (2.5%). Remarkably, the presence of heparin-dependent antibodies in these nine patients (six of whom were previously exposed to heparin) was not associated with thrombocytopenia except in two cases. The frequency of heparin-dependent antibodies as detected by a functional assay (HIPA) in our study was 8.4% (30/358 patients, none of whom was thrombocytopenic). This finding is in accordance with the incidence reported by Warkentin et al (1995) using another functional test (SRA) in patients on prophylactic-dose heparin. There is no clear explanation for the discrepant test results between HIPA assay and PF4/hep Elisa. Others have suggested that both tests would recognize antibodies directed against different target antigens (Greinacher et al, 1994). For the practical clinical situation, no conclusions can be drawn about the predictive value of both tests.

The clinical significance of heparin dependent antibodies is unknown as is their incidence and the period during which they are detectable in plasma of patients without clinical signs of HITT. According to one report the antibodies disappeared from the plasma after 2 months in two patients, whereas in another patient the antibody was still demonstrable 3 years after the occurrence of heparin-induced thrombocytopenia (Fabris et al, 1995). However, long-term existence of antibodies after previous heparin treatment, cannot explain the presence of antibodies in our patient group since all day 0 test samples were negative. Moreover, the long mean interval from last heparin exposure makes this even more unlikely.
In conclusion, the frequency of HITT in the category of patients studied was lower than expected on the basis of previous analyses and is in contrast to recently observed higher frequencies in patients undergoing orthopedic or cardiovascular surgery (Doubine et al, 1995; Warkentin et al, 1995). The clinical relevance of the presence of HITT antibodies in non-symptomatic, non-thrombocytopenic patients is as yet unknown.

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CHAPTER 3

THE VALUE OF ALLOANTIBODY DETECTION IN PREDICTING RESPONSE TO HLA MATCHED PLATELET TRANSFUSIONS

Mark-David Levin[1], Mies Kappers-Klunne[1], Kees Sintnicolaas[2], Bronno van der Holt[3], Huub H.D.M van Vliet[1], Bob Löwenberg[1], Mars B. van 't Veer[1]

[1]Department of Hematology, Erasmus MC, Rotterdam,
[2]Sanquin Blood Bank South West Region, Rotterdam,
[3]Department of statistics, Erasmus MC, Rotterdam.

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SUMMARY

Alloantibody tests demonstrate immunological causes of insufficient increments in random platelet transfusions. The value of a positive or negative test result in predicting outcome of human leucocyte antigen (HLA)-matched transfusions in patients refractory to leukodepleted random platelet transfusions has not been assessed.

We retrospectively evaluated the outcome of the first HLA-matched platelet transfusion in 72 patients with haematological diseases in two ways: first the strategy according to which the patient was selected for HLA-matched platelet transfusions was analysed. The strategies were: (I) results of alloantibody tests were not available; (II) a positive alloantibody test; (III) a negative alloantibody test. Secondly, the outcome of the first HLA-matched transfusion was investigated relative to the results of alloantibody tests, irrespective the decision strategy.

No significant association was found between the decision strategy and the outcome of the first HLA-matched platelet transfusion. Positive alloantibody tests, however, predicted a better outcome of the first HLA-matched platelet transfusion ($P = 0.04$ and $P = 0.03$ after 1 and 16 h, respectively).

In patients refractory to random platelet transfusions, positive alloantibody tests predict a better outcome of HLA-matched platelet transfusions. Patients with negative alloantibody tests, however, may benefit from HLA-matched platelet transfusions.

INTRODUCTION

Patients with hematological illnesses and thrombocytopenia following cytotoxic therapy frequently require platelet transfusions in order to prevent or treat bleeding complications. Usually pooled platelet suspensions from random donors are used. Most of these random platelet transfusions are matched for the ABO-blood group because high titers of antibodies against ABO may enhance platelet destruction and ABO-blood group matching is relatively easy (Duquesnoy et al, 1979; Lee & Schiffer, 1989; Carr et al, 1990). Donor platelets, however, are not routinely matched for human leukocyte antigens (HLA) or human platelet antigens (HPA), because of the low prevalence of antibodies against HLA- or HPA-antigens and difficulties in finding HLA- or HPA-matched platelet suspensions (Bishop et al, 1988, 1991; Legler et al, 1997). Antibodies against HLA- and HPA-antigens can cause platelet refractoriness and febrile transfusion reactions. In order to establish whether poor platelet recovery is caused by immunological (antibodies against HLA- or HPA-antigens) or by non-immunological factors (e.g. splenomegaly, older transfused platelets, fever, drugs, bleeding) a variety of tests are used to demonstrate the presence of alloantibodies (Mittal et al, 1968; von dem Borne et al, 1978; Horai et al, 1981; Schiffer & Young, 1983; Santoso et al, 1984; Court & LoBuglio, 1986; Lazarchick & Hall, 1986; van der Velden et al, 1986; Kiefel et al, 1987a,b: Sintnicolaas et al, 1987; Freedman et al, 1988; Pamphilon et al, 1989; Freedman & Hornstein, 1991; Sintnicolaas et al, 1991; Worfolk & MacPherson, 1991; Zachary et al, 1995, 2001; Köhler et al, 1996; Lucas et al, 1997; Gratama et al, 1998; Lubenko & Rodi, 1998; Levin et al, 1999; Moses et al, 2000; Kurz et al, 2001; Lubenko et al, 2001; Worthington et al,

2001; Levin et al, 2003a). These tests have been demonstrated to be of use in predicting a loss of response to random donor platelet transfusions in alloimmunized patients. The value of alloantibody testing in predicting the success of an HLA-matched platelet transfusion in patients refractory to random donor platelet transfusions, however, has not been established.
In order to evaluate the importance of alloantibody tests in assigning patients to HLA-matched transfusions we conducted a retrospective study in which three decision strategies (associated with distinct clinical contexts) and the results of alloantibody tests were assessed with regard to the platelet recovery of the first HLA-matched platelet transfusion. The effect of patient variables and transfusion characteristics that may have influenced platelet recoveries were also studied in all HLA-matched platelet transfusions that patients received.

METHODS AND MATERIALS

Patients

From January 1997 to January 2002, 89 patients received 619 HLA-matched platelet transfusions at the department of hematology, Erasmus Medical Centre. Seventeen patients (19%), receiving 59 HLA-matched platelet transfusions, were excluded from the analysis because of a non-hematological diagnosis in nine (testicular carcinoma in one, ovarian carcinoma in three and surgery in five) and missing data in eight patients. A total of 560 HLA-matched platelet transfusions (91%) in 72 patients (81%), average eight HLA-matched platelet transfusions per patient (range 1-36), were studied (Table 1).

Tabel 1. Patient characteristics.

Characteristics:	Category:	Number:
Sex	Male	22
	Female	50
Diagnosis	ALL	4
	AML	29
	CLL	1
	CML	4
	MM	8
	NHL	2
	MDS	10
	AA	9
	ITP	3
	Other	2
Treatment	Chemo-/other therapy	49
	Auto SCT	5
	Allo SCT	18

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CLL = chronic lymphatic leukemia; CML = chronic myeloid leukemia; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; MDS = myelodysplastic syndrome; AA = aplastic anemia; ITP = idiopathic thrombocytopenic purpura; Auto SCT = autologous stem cell transplantation; Allo SCT = allogeneic stem cell transplantation.

Decision strategies

Patients that demonstrated platelet recovery (defined below) of less than 20% after 1 h following at least two consecutive platelet transfusions (refractory) and in whom an immune mediated cause was presumed (non-immunological causes of platelet refractoriness were absent, patients developed chills or fever following random platelet transfusions), were selected for HLA-matched platelet transfusions. Three clinical contexts that led to the decision to transfuse HLA-matched platelets were distinguished: (I) transfusion without an alloantibody test result [lymphocyte immunofluorescence test (LIFT) or monoclonal antibody-specific immobilization of platelet antigens (MAIPA) result became known after the HLA-matched platelet transfusion]; (II) transfusion following a positive alloantibody test result; (III) transfusion despite a negative alloantibody test result (an empiric HLA-matched platelet transfusion was given). The outcome of these three transfusion strategies were compared retrospectively.

Platelet concentrates

Platelet concentrates were obtained from the Sanquin Blood Bank South West Region and were prepared as an apheresis product from a single HLA-matched volunteer donor consisting of a wide range of platelets ($82 – 1173 \times 10^9$) per transfusion. The HLA-match was classified as *compatible*, when donor and recipient shared all HLA-A and B antigens or when the donor expressed less HLA-A and B antigens than the recipient. It was designated as *cross-reacting*, when cross-reactive HLA-mismatches between the donor and recipient HLA-A and B antigens were present (Duquesnoy et al, 1979). The platelet concentrates were filtered through a PLX-5 Asahi filter (Baxter, La Chatre, France) to deplete the suspension of leucocytes. The average leukocyte number was 0.05×10^6 per platelet concentrate (range $0.00\text{-}0.20 \times 10^6$). In patients that received a hematopoietic stem cell transplantation the platelet concentrates were irradiated with 25 Gy in order to prevent graft versus host disease (GvHD) by transfused lymphocytes (Slichter, 1997). The platelet concentrates were stored at 22^0 C under continuous agitation for a maximum of five days. Also erythrocyte concentrates were always leukocyte depleted and irradiated for the same reasons as stated above.

Platelet transfusion results

$$\text{Percentage recovery} = \frac{\text{platelet increment } (10^9/\text{l}) \times \text{blood volume (l)}}{\text{number of transfused platelets } (10^{11})} \times 100$$

A successful platelet transfusion was defined as a recovery of platelets of 20% or more after 1 h or 10% or more 16 h after transfusion (Davis et al, 1999; Novotny, 1999).

Tests

Assays for HLA-antibodies

I. Indirect Lymphocyte Immunofluorescence Test.

A slightly modified method was used according to the method described elsewhere (Sintnicolaas et al, 1991; Gratama et al, 1998; Levin et al, 2003a). Briefly, four-parameter flow cytometry was performed on a lymphocyte population from a panel of five donors after incubation with patient's serum and a diluted mixture of fluorescence-conjugated goat anti-immunoglobulin G (IgG). The donor panel was simultaneously incubated with a positive and negative control serum in order to detect inter assay variation. The patient was considered to be sensitized against a donor if the fluorescence showed more than 36% of all lymphocytes exceeding a standard threshold (determined by more than 100 negative controls). A patient was considered immunized against a broad range of HLA-antigens (alloimmunized) if 40% or more of all five donors showed a positive result. The LIFT measures mainly antibodies against HLA-antigens.

II. Monoclonal antibody-specific immobilization of platelet antigens.

This test was performed according to Kiefel et al (1987b) with slight modifications. Briefly, platelet-rich plasma (PRP) was stored and frozen after fixation with paraformaldehyde (Freedman & Hornstein, 1991) from a pool of 100 donors. After defrosting the PRP, the platelets were solubilized with Tris-buffered saline Nonidet P40 (BDH, Poole, Dorset, England) after successive incubation with patient serum and a monoclonal mouse anti-human antibody against the major histocompatibility complex (MHC)-class I molecule (Dako, Glostrup, Denmark). The solubilized platelets were added to the wells of a microtitre plate that was covered with a polyclonal rabbit anti-mouse immunoglobulin (Dako). After incubation and washing the microplates a horseradish peroxidase-conjugated rabbit anti-human antibody against immunoglobulin G was added. After a further incubation and washing, ABTS (2,2'-azinobis 3-ethylbenzthiazolinesulphonic acid)-peroxide (Roche, Basel, Switzerland) was added and the absorbance was measured. The absorbance of a negative panel serum was subtracted from the absorbance of patient's serum. When this subtraction exceeded 150 (two times the standard deviation, as was previously determined by more than 20 negative donors) the patient was considered to be immunized against a broad range of HLA-antigens (alloimmunized). This test detects antibodies against HLA-antigens.

Indirect Platelet Immunofluorescence Test.

This method was used with slight modifications to the methods of two different techniques described previously (Freedman & Hornstein, 1991; Levin et al, 2003a). Briefly, four-parameter flow cytometry was performed on a panel platelet population after incubation with patient serum and fluorescence-conjugated goat anti-IgG. The panel platelet population was derived from the fresh whole blood of five individual donors (Levin et al, 2003a) or from platelets (stored and frozen as PRP) after fixation with paraformaldehyde (Freedman & Hornstein, 1991) of a pool of 100 donors. In the first technique a donor was considered to be positive when more than 17% of all platelets exceeded a standard threshold fluorescence (established in more than 100 negative controls). A patient was considered immunized against

a broad range of HLA-antigens if 40% or more of all donors showed a positive result. In the second technique a patient was considered immunized against a broad range of HLA-antigens if more than 20% of all platelets exceeded a specific threshold (previously determined on 20 negative controls). The platelet immunofluorescence test (PIFT) measures all causes of IgG bound to platelets (antibodies against HLA- and platelet-specific antigens, autoantibodies and possibly immune complexes).

Patient variables and transfusion characteristics

The recovery after 1 and 16 h of all HLA-matched platelet transfusions that the study group received were compared to patient variables sex, diagnosis and therapy (described in Table 1) and transfusion characteristics ABO-match and HLA-match.

Statistical methods.

The association between strategies and recovery after the first transfusion or the test results and recovery after the first transfusion was investigated using Fisher's exact test in case of discrete variables, and the Kruskal-Wallis test in case of continuous outcomes. Univariate logistic regression with adjustment for patients with multiple transfusions was used to evaluate the association between the patient and transfusion characteristics and the recovery in all transfusions. All P-values are two-sided and a significance level of 0.05 was used.

RESULTS

Alloantibody results of the study group

Test results in 72 patients were as follows: 13 patients had negative PIFT and HLA-tests (LIFT or MAIPA) and 51 patients had positive PIFT and HLA-tests. In five patients the PIFT was positive and the HLA-tests were negative, in three patients the PIFT was negative and the HLA-tests were positive.

Response to HLA-matched platelet transfusions according to strategy

Of the 72 patients, 17 received HLA-matched transfusions following strategy I (transfused without an alloantibody test result), 39 following strategy II (transfused following a positive alloantibody test) and 15 following strategy III (transfused following a negative alloantibody test) (Tabel 2). In one patient it was not clear whether the decision was based upon a positive test result. This patient was excluded from this analysis, but has been included in the analysis on test results irrespective of strategy. The average recovery of the first HLA-matched platelet transfusion did not differ significantly between the three strategies. The average recoveries (± SD) of the transfusions after 1 h were 42% (± 18%), 47% (± 26%) and 35% (± 27%) for strategies I, II and III (P = 0.17) and the average recoveries after 16 h were 20% (± 20%), 32% (± 25%) and 17% (± 14%) (P = 0.10) respectively (Table 2). Successful transfusions for strategies I, II and III were noted in 94%, 85% and 73% after 1 h (P = 0.27) and 76%, 77% and 67% after 16 h (P = 0.71), respectively.

Response to HLA-matched platelet transfusions according to test results

Of the 72 patients, 54 had positive HLA-tests (14 strategy I, 39 strategy II and one not clear). In 18 patients (three strategy I and 15 strategy III) no HLA-alloantibodies could be detected by these techniques (LIFT and MAIPA). The average platelet recovery of the first HLA-matched platelet transfusion in patients with a positive HLA-test after 1 hour was 47% (± 24%) and in patients with a negative test 35% (± 25%) (P = 0.04). The average platelet recovery after 16 h of the first HLA-matched platelet transfusion in patients with a positive test was 31% (± 24%) and 15% (± 14%) in patients with a negative test (P = 0.03) (Table 3). Successful transfusions for patients with a positive and negative test were present in 87% and 78% after 1 h (P = 0.45) and in 80% and 61% after 16 h (P = 0.13), respectively.

Tabel 2. Decision strategy versus platelet recovery.

	Strategy			*P-value*
	I N = 17	II N = 39	III N = 15	
Positive HLA-test	14	39	0	-
$Recovery_{1h}$	42% ± 18%	47% ± 26%	35% ± 27%	0.17
$Recovery_{16h}$	20% ± 20%	32% ± 25%	17% ± 14%	0.10
$Successful\ transfusions_{1h}$	16 (94%)	33 (85%)	11 (73%)	0.27
$Successful\ transfusions_{16h}$	13 (76%)	30 (77%)	10 (67%)	0.71

The number of patients with a positive HLA-test (LIFT or MAIPA), the recovery (average ± SD) after 1 and 16 h and the number (percentage) of successful transfusions after 1 (recovery ≥ 20%) and 16 h (recovery ≥ 10%) of the first HLA-matched transfusion compared to strategy I (test not available), II (positive test) and III (negative test). One patient is not included in this table because the decision strategy was not clear.

Tabel 3. HLA-test results vs. platelet recovery.

	HLA-test		*P-value*
	Positive N = 54	Negative N = 18	
$Recovery_{1h}$	47% ± 24%	35% ± 25%	0.04
$Recovery_{16}$	31% ± 24%	15% ± 14%	0.03
$Successful\ transfusions_{1h}$	47 (87%)	14 (78%)	0.45
$Successful\ transfusions_{16h}$	43 (80%)	11 (61%)	0.13

The recovery (average ± SD) after 1 and 16 h and the number (percentage) of successful transfusions after 1 (recovery ≥ 20%) and 16 (recovery ≥ 10%) compared with a positive or negative HLA-test (LIFT or MAIPA).

In this series a positive PIFT was found in 56 patients and a negative PIFT in 16 patients. The average platelet recovery after 1 h of the first HLA-matched platelet transfusion in patients

with a positive PIFT was 46% (± 25%) and in patients with a negative test 38% (± 24%) (P = 0.14). After 16 h the average platelet recovery of the first HLA-matched platelet transfusion in patients with a positive PIFT was 30% (± 24%) compared with 17% (± 14%) in patients with a negative test (P = 0.08). Successful transfusions for patients with a positive and negative PIFT were present in 86% and 81% of cases after 1 h (P = 0.70) and in 79% and 75% of cases after 16 h (P = 0.21) respectively.
In the three patients with a positive HLA-test and a negative PIFT, a successful increment was established in all of the first HLA-matched transfusions both after 1 and 16 h. Among the five patients with a negative HLA-test and a positive PIFT, 80% of first HLA-matched transfusions resulted in a successful recoveries at 1 and 16 h after transfusion.

Analysis of patient and transfusion factors
With regard to the recovery values after 1 and 16 h of the 560 HLA-matched transfusions, no differences were noted between male and female patients. There was no apparent impact of diagnosis or treatment (chemotherapy compared with autologous or allogeneic stem cell transplantation) on the values of platelet recovery either. ABO-blood group incompatibility did not predict platelet recovery, although a trend towards lower recovery 16 h after transfusion (P = 0.07) was evident (83% of successful outcomes in ABO compatible HLA-matched transfusions compared with 69% in ABO incompatible HLA-matched transfusions, in seven transfusions the ABO-match was not known). In 145 transfusions with a cross-reactive HLA-mismatch 77% of successful outcomes were established after 1 h, which was significantly less (P = 0.04) than the 88% of successful outcomes in 404 HLA-compatible transfusions. No difference in recoveries after 16 h between cross-reactive and compatible transfusions was found, 82% of successful transfusions in compatible transfusions and 77% in incompatible transfusions (P = 0.44). In 11 of the 560 transfusions no HLA match could be found retrospectively.

DISCUSSION

Alloantibody measurement in the serum of patients not responding to random donor platelet transfusions has become common practice. Several studies have demonstrated that alloantibody tests predict for an insufficient increment of random non-leucodepleted platelet transfusions (Bishop et al, 1988, 1991; Legler et al, 1997). Previous studies have not found a correlation between test result and platelet recovery of random transfusions (Doughty et al, 1994; Ishida et al, 1998; Levin et al, 2003b). The importance of these tests in predicting the success of HLA-matched platelet transfusions in patients refractory to leucodepleted random donor platelet transfusions has not been studied.
In this retrospective study, three decision strategies (not based on an available alloantibody test result; based on a positive alloantibody test; and despite a negative alloantibody test) of selecting patients for HLA-matched transfusions were compared. We could not demonstrate a difference between these three strategies in regard to the increment of the first HLA-matched platelet transfusion. A trend, however, towards a lower average transfusion result was observed for strategy III. We may have been unable to demonstrate a significant difference

between these three strategies because of the limited number of patients accrued during the 5 year period. In addition, 82% of the 17 transfusions following strategy I demonstrated a positive HLA-test in retrospect, and thus the majority of patients of both strategy-groups I and II showed serological evidence of alloimmunization.

In the second part of the analysis, the results of a positive or negative HLA-test were related to the recovery of the first HLA-matched transfusion. Significantly better average recoveries of the first HLA-matched platelet transfusion were observed in the patient group with a positive HLA-test. This underscores the importance of patient selection with the help of an HLA-test for HLA-matched transfusions, in a population receiving leucodepleted blood products. However, seven of 54 patients with a positive HLA-test showed a poor recovery of the first HLA-matched transfusion suggesting a possible role of non-immune factors. These factors had not been specified or recognized by the treating physician as the cause of refractoriness, and therefore their nature remains unclear. On the contrary, it is of note that 14 of 18 patients who demonstrated a negative HLA-test showed positive response to HLA-matched transfusions. The adequate recovery of HLA-matched platelet transfusions in the absence of documented HLA sensitization cannot easily be explained but it is possible that transient non-immunological factors have played a role in the lack of response of these patients to earlier random platelet transfusions. In addition, it is conceivable that the restricted sensitivity of HLA-tests in detecting alloantibodies is too restricted in a proportion of patients. Finally in vivo adsorption of alloantibodies to transfused platelets at the time of serum sampling for the alloantibody test might have interfered with the results.

The results of HLA-tests and PIFT demonstrated a considerable overlap. Therefore, the added value of PIFT in the series as a predictor of immunization was limited. The PIFT did not demonstrate a significant correlation between test result and the average recovery of the first HLA-matched platelet transfusion, although a trend towards poorer recoveries in patients with a negative PIFT was noted. The discrepant results (11%, eight of 72) between HLA-tests and PIFT may be explained by the fact that HLA-tests only measure alloantibodies against HLA-antigens and the PIFT also detects other alloantibodies (platelet-specific, autoantibodies and possibly immune complexes). In addition HLA-antigen expression on platelets is less than that on lymphocytes, which are used as targets in the LIFT. This may explain why the HLA-tests demonstrated a stronger association with increment of HLA-matched platelet transfusions than the PIFT. Furthermore, the incidence of platelet specific antibodies with or without the presence of HLA antibodies is low (Sanz et al, 2001). Platelet specific antibodies, therefore, are not expected to influence the outcome of this analysis.

In looking for other factors interfering with platelet recovery we considered the total 560 HLA-matched transfusions in the study group. No differences in platelet recoveries were found in relation to sex, diagnosis or therapy. We were not able to demonstrate a significant effect of ABO-incompatibility upon platelet recovery of HLA-matched transfusions. Although the study was not assigned to primarily address this issue, this is in contrast to other reports on random platelet transfusions (Duquesnoy et al, 1979; Lee & Schiffer, 1989; Carr et al, 1990; Heal et al, 1993). Our patients received random ABO-matched platelet transfusions prior to HLA-matched transfusions. This may have avoided a boost to ABO-antigens. We demonstrated a significantly better recovery after 1 h of a compatible HLA-match in contrast

to a cross-reactive HLA-match. This emphasizes the importance of the effort that has to be undertaken to find the best match for a patient immunized against a broad range of HLA-antigens.
In conclusion a positive HLA-test predicts for better overall recovery of HLA-matched platelet transfusions in patients refractory to random donor platelet transfusions. Patients refractory to random donor platelet transfusions with a negative HLA-test and without non-immunological factors present, however, may benefit from HLA-matched transfusions, although the average recovery is considerably lower. In these patients or in patients in whom no results of HLA-tests are available an empiric HLA-matched platelet transfusion may be considered.

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CHAPTER 4

CYCLOSPORIN A FOR THE TREATMENT OF PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA REFRACTORY TO CORTICOSTEROIDS OR SPLENECTOMY

M.C. Kappers-Klunne, M.B.van ‘t Veer

Department of Haematology, University Hospital Rotterdam, the Netherlands

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SUMMARY

Patients with chronic immune thrombocytopenic purpura (ITP) who are unresponsive to corticosteroids require splenectomy, but if this fails, treatment is difficult. We tried to induce durable remissions in ITP patients refractory to corticosteroids before or after splenectomy by applying strong immunosuppression with the combination of cyclosporin A (CyA 5 mg/kg/d) and prednisone (0.4 mg/kg/d). Patients were assigned to one of two groups. Group 1, 10 patients refractory to prednisone; and group 2, 10 patients refractory to at least prednisone and splenectomy. Overall response rate was 55% (50% in group 1 and 60% in group 2 patients). Nine of the 10 patients in group 1 finally had a splenectomy because of relapse, insufficient response or toxicity of CyA. Thirty percent of the patients discontinued CyA because of side-effects; hypertension, severe headache and muscle pain being the most frequent encountered. It is concluded that CyA treatment does not avoid, but only postpones splenectomy in chronic ITP patients who are refractory to corticosteroids. However, CyA can be useful in a subgroup of patients with corticosteroid- and splenectomy-refractory ITP, but treatment toxicity is high.

INTRODUCTION

Chronic idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by platelet destruction caused by an antiplatelet autoantibody resulting in platelet phagocytosis in the mononuclear phagocytic system (Karpatkin, 1997).
ITP is classically defined by a decreased number of circulating platelets and a normal to supernormal bone marrow megakaryocyte mass occurring in the absence of agents or systemic diseases known to induce thrombocytopenia.
Glucocorticosteroids have been accepted as initial treatment for ITP, although only a third of the patients will have a long-term response. Splenectomy is the treatment of choice for patients failing on corticosteroids. This results in a much higher cure rate than any medical regimen, with sustained complete and partial response rates of 60 % and 12% respectively (Berchtold & McMillan, 1989). However, in older patients response rates may be much lower (Guthrie et al, 1988; Cortelazzo et al, 1991).
Treatment is regarded unnecessary in patients with a platelet count of >40 x 10^9/l. Severe bleeding episodes have only been recorded at platelet counts less than 30 x 10^9/l with an increased incidence in patients aged >60 years (Cortelazzo et al, 1991). Thus, in patients failing to respond to corticosteroids and splenectomy with a platelet count of <30 x 10^9/l, further treatment should be considered. Therapeutic options for these patients include a wide range of treatment regimens such as immunosuppression with high-dose methylprednisolone, cyclophosphamide, azathioprine or vinca-alkaloids; blockade of the mononucleair-phagocytic system with high dose gamma-globulin or IgG anti-Rh (D) and miscellaneous regimens such as: danazol, ascorbic acid, staphylococcal protein-A plasmapheresis, interferon-α (McMillan, 1997) and very recently, monoclonal antibodies (Bussel et al, 1999; Periotta et al, 1999) and even autologous stem cell transplantation (Lim et al, 1997). Treatment results generally have been disappointing.

In ITP the precise mechanism of auto-antibody formation is unknown, but more recently T cell dysfunction has been suggested as the most important causative factor rather than abnormal B cell function as previously was assumed (Mylvanganam et al, 1988; Ware & Howard, 1993).
Cyclosporin A (CyA) is a powerful immunosuppressant (Denton et al, 1999; Kahan, 1989). CyA selectively inhibits both antigen-induced activation of $CD4^+$ lymphocytes and the production by these cells of interleukin 2 (IL-2) and other cytokines. This action results in an indirect inhibitory effect on the growth and differentiation of B lymphocytes.
In view of its action it may be expected that CyA could induce and, especially maintain, remission in autoimmune disorders, particularly in those with mechanisms mediated by T cells. The drug has proven effectiveness in psoriasis and other autoimmune diseases (Ellis et al, 1991; Wells & Tugwell, 1993).
Corticosteroids have both immunosuppressive and non-specific anti-inflammatory properties. They inhibit IL-1 generation, thereby potentiating the action of cyclosporin on helper T cells (Kahan, 1989). The strong immuno-suppressive effect of the combination of CyA and corticosteroids is potentially very effective in arresting the immune response. Therefore, we initiated a prospective study aimed at attaining a durable remission with this regimen in patients refractory to corticosteroids alone or to corticosteroids and splenectomy.

PATIENTS AND METHODS

Patients.
Patients who presented to the University Hospital Rotterdam and some affiliated hospitals were included in the study if they fulfilled the following criteria: chronic ITP defined as isolated thrombocytopenia, diffuse intravascular coagulation and pseudothrombocytopenia being excluded; absence of splenomegaly as documented by ultrasound sonography and normal bone marrow morphology with normal or increased numbers of megakaryocytes; refractoriness to conventional-dose prednisone as initial treatment or to both prednisone and splenectomy; serum creatinine <120 μmol/l; informed consent. The protocol was approved by the medical ethical committee of our hospital.

Treatment.
Patients were assigned to one of two groups.
Group 1 comprised chronic ITP patients or patients presenting with a relapse after a remission of > 6 months, who were refractory to prednisone (1.5 mg/kg/day) for 3 weeks. At this point, prednisone was tapered to zero within 2-4 weeks and oral CyA was started 6mg/kg/day orally (in two doses) and continued for at least 4 weeks. If complete response (CR) had been attained, CyA was tapered by 50 mg/day every 2 weeks. If by 4 weeks the patient was in partial response (PR), CyA was continued until maximum platelet counts were reached, but no longer than 3 months. At the point of maximum platelet count, CyA was tapered in the same way as for patients who had reached CR. If after 4 weeks of CyA, the platelet count still

was or had returned to < 40 x 10^9/l, the patient was regarded as no response (NR) and CyA was stopped and the patient proceeded to splenectomy.
Group 2 patients were refractory to at least prednisone and splenectomy. Patients started on CyA 5 mg/kg/day (in two doses) in combination with prednisone 0.4 mg/kg/day. In case of CR, CyA was tapered in the same way as in group1 patients. If, by 12 weeks PR was reached, CyA and prednisone were continued until maximum platelet count, but not longer than 4.5 months. At that point CyA was stopped and prednisone was gradually tapered and stopped. In case of NR at 12 weeks, CyA and prednisone were stopped. In all patients CyA treatment was monitored by weekly clinical and laboratory examination during the first 4 weeks, after which the interval between monitoring visits was extended, dependent on the patients tolerance of treatment.

Response criteria.
Complete response (CR), platelet count > 110 x 10^9/l for more than 12 weeks. Partial response (PR), platelet count > 40 x 10^9/l for >8 weeks. No response (NR) platelet count always < 40 x 10^9/l. Transient response (TR), platelet count > 40 x 10^9/l for >2 but less than 4 weeks.

Toxicity.
Assessment of toxicity included regular monitoring of clinical features, such as blood pressure, paresthesias, gastrointestinal complaints, gingival hypertrophy, headache and determination of whole blood or plasma CyA levels and serum creatinin. Initially, CyA concentration was determined on plasma samples using a monoclonal antibody radioimmuno assay. Later, CyA levels were determined in whole blood with the EMIT 2000-CsA immunoassay (Dade Behring, San Jose, Ca, USA). In one patient a polyclonal antibody assay was used (Abbott TDX, Hoofddorp, The Netherlands) to measure blood CyA concentration.

RESULTS

Treatment results.
Twenty patients were included in the study, 10 patients in group 1 and 10 patients in group 2.
In group 1 (Table I) CR was reached in three cases. In patient 1, CR lasted for 2 years off treatment; a relapse was triggered by infection. In patient 3, CR lasted for 2 years off treatment until a spontaneous relapse occurred. Patient 7 reached CR but, after stopping CyA, remained dependent on low-dose prednisone (5 mg/d) to maintain safe platelet levels. Two patients in group 1 (patients 2 and 4), reached PR lasting 324 and 95 d, respectively, at which time CyA had to be stopped because of side-effects and the patients proceeded to splenectomy. Two patients (patients 5 and 8) attained TR and three cases (patients 6, 9 and 10) qualified as NR. In patient 6, CyA was started when his platelet count was 140 x 10^9/l because of preceding high-dose prednisone. After starting CyA, his platelet count further increased to 166 x 10^9/l, but quickly decreased to <20 x 10^9/l thereafter on tapering prednisone, so he was regarded a NR. Nine patients ultimately underwent splenectomy

because of relapse or insufficient response. Splenectomy resulted in durable remission in all patients in group 1 for a mean follow-up of >2 years.
Two patients in group 2 (Table II) were in continuing CR lasting for >4 (patient 11) and >2 years (patient 12), respectively, and a third (patient 17) who initially reached CR, converted to PR lasting for >1 year off treatment. Three patients (patients 15, 16 and 18) reached PR. In two cases, CyA was stopped (after 91 and 31 days of CyA respectively) because of side-effects. Four cases (patients 13, 14, 19 and 20) in this group were NR.

Toxicity.
Mean plasma/blood CyA levels always remained within the therapeutic range except in four patients (patients 7, 9, 12 and 14). Remarkably, patient 12 reached long-term CR despite sub-optimal plasma CyA levels. Patient 15 attained long-term PR on mean blood CyA levels above target values. Despite optimal or sometimes even suboptimal CyA levels, the majority of the patients experienced side effects. Hypertension, occurring in 6 cases and severe muscle pain and/or headache in seven cases were the most frequently encountered side effects. Discontinuation of CyA was deemed necessary in six patients (patients 2, 4, 7, 9, 14 and 16). Reasons for stopping are shown in Tables IB and IIB.

DISCUSSION

In this prospective study we examined the feasibility of intensive immunosuppression using the combination of CyA and corticosteroids to induce remission in ITP patients refractory to corticosteroids before or after splenectomy. Based on the assumption of a T cell-mediated mechanism for aberrant B-cell activity in ITP (Mylvanganam et al, 1988), combining CyA with corticosteroids may result in more profound immunosuppression.
Reports on therapeutic measures after failure of initial corticosteroid treatment to prevent splenectomy in chronic ITP (group 1 patients) are scarce and concern only small patient numbers or incidental cases. Schultz et al (1995) described the results of CyA treatment in five children with ITP who were refractory to intravenous immunoglobulin and prednisone, with two of them also unreactive to anti-Rhesus D immune globulin. The CyA dose in this study was 5 mg/kg/d, which was increased to 10 mg/kg/d in cases of NR after 2 weeks. Responses were minimal and transient, lasting no longer than 4 weeks at the cost of intolerable toxicity, which probably in part, has to be ascribed to the relatively high CyA levels required to obtain an increase in platelet count in these children. The toxicity observed in this study is in accordance with our own experience but the response seemed to be better in our patients.
In our study, 3 of the 10 patients in group 1 reached CR, all lasting >1 year off treatment. The two partial responders in this group had to discontinue CyA because of side-effects. Ultimately, all but one patient in this group had a splenectomy because of relapse. Thus, CyA merely postponed splenectomy but could not prevent it.
CyA has also proven to be effective in refractory ITP (group 2 patients). The first reports concern only anecdotal cases and doses used varied widely as did treatment results (Kelsey et al, 1985; Miescher & Beris, 1985; Velu et al, 1987; Matsumara et al, 1988). Siegel et al

Table I. (A and B). Cyclosporin A (CyA) in ITP patients refractory to corticosteroids (group 1)

A						
Patient	**age (years) / sex**	**days from diagnosis**	**period of CyA (d)**	**CyA dose (mg / kg)**	**response**	**concomitant treatment**
1.	35 / M	180	136	6	CR	pred 1mg/kg/d
2.	69 / M	21	324	6-7.5	PR	pred 0.8 mg/kg/d
3.	64 / M	1380	83	2.5-5	CR	pred 0.3 mg/kg/d
4.	18 / F	64	95	6-8	PR	pred 1 mg/kg/d
5.	53 / F	24	46	6	TR	none
6.	30 / M	243	137	6	NR	pred 0.5 mg/kg/d
7.	84 / F	21	101	6	CR	pred 1 mg/kg/d
8.	52 / M	39	21	5	TR	pred 0.4 mg/kg/d
9.	38 / F	238	9	5	NR	pred 0.15 mg/kg/d
10.	55 / M	62	42	6	NR	none

CR, complete remission; PR, partial remission; NR, no response; TR, transient response; pred, prednisone.

B							
Patient	**platelets (x 10^9/l)**		**days from start CyA to**		**CyA levels***		**toxicity**
	start	**maximum count**	**platelets >100 x 10^9/l**	**maximum count**	**mean**	**median**	
1.	8	269	6	52	75[1)]	73	hypertension
2.	3	107	234	234	75[1)]	64	fatigue, nausea, gum hyperplasia → stop CyA
3.	8	253	42	83	275	260	hypertension
4.	36	73	n.a.	52	165	140	fatigue, muscle ache, burning → stop CyA
5.	35	45	n.a.	4	274	240	muscle ache, mild/transient serum creatinin ↑
6.	140	166	n.a.	26	241	187	none
7.	30	187	21	101	310	320	hypertension, peripheral edema → stop CyA
8.	3	51	n.a.	15	155	175	none
9.	4	36	n.a.	4	100	n.a.	head/muscle ache, hypertension → stop CyA
10.	n.a.	8	n.a.	n.a.	189	210	gum hyperplasia

n.a., not applicable; *target levels: 150-300 ng/ml; [1)]40-80 ng/ml

Table II. (A and B). Cyclosporin A (CyA) in ITP patients refractory to at least prednisone and splenectomy (group 2).

(A)

Patient	age (years) / sex	previous treatment*	time from splenectomy (years)	period of CyA (days)	CyA dose (mg / kg)	response	concomitant treatment
11.	60/F	P, IVIG, S	0.3	277	5	CR	pred* 0.4 mg/kg/d
12.	47/F	P, S	1.6	196	7	CR	pred 0.4 mg/kg/d
13.	29/M	P, IVIG,S	0,07	60	5-7.5	NR	pred 0.6 mg/kg/d
14.	36/F	P, S, IVIG, vitC, danazol, im	3.2	31	6	NR	none
15.	51/M	P, IVIG, S	1.9	153	7	PR	pred 0.2 mg/kg/d
16.	50/F	P, S	12	91	5	PR	none
17.	55/F	P, HD, S	0.8	227	5	CR	pred 0.4 mg/kg/d
18.	34/F	P, S, HD	6.4	124	5	PR	none (HD immediately preceding CyA)
19.	66/M	P, IVIG, S, im, dap, vinc	0.7	74	4 -5	NR	pred 0.3 mg/kg/d, intermittent IVIG
20.	49/M	P, S, im, IVIG	0.5	56	3	NR	none

*P / pred, prednisone; IVIG, intravenous immunoglobulin; HD, high dose dexamethasone; S, splenectomy; im, immuran; vinc, vincristin; dap, dapsone; vitC, vitamin C

(B)

patient	platelets (x10^9/l)		days from start CyA to		CyA levels		toxicity
	start	maximum count	platelets > 100 x10^9/l	maximum count	mean	median	
11.	10	415	6	172	160	n.a.	hypertension
12.	24	254	8	28	16[1)]	19	severe muscle ache, paraesthesias
13.	3	n.a.	n.a.	n.a.	64[1)]	68	none
14.	4	6	n.a.	n.a.	10[1)]	n.a.	severe headache → stop CyA
15.	20	135	17	45	102[1)]	86	hypertension
16.	20	60	n.a.	61	233	215	head/muscle ache, ↑serum creatinin → stop CyA
17.	22	313	83	147	555[2)]	551	hypertension
18.	6	78	n.a.	43	150	150	nausea
19.	19	n.a.	n.a.	n.a.	222	225	severe muscle ache, paresthesias
20.	20	n.a.	n.a.	n.a.	250	188	head/muscle ache

n.a., not applicable; *target levels: 150-300 ng/ml; [1)]40-80ng/ml; [2)]300-800 ng/ml.

(1991) observed 7 responses in 9 patients with platelets under 50 x 10^9/l. Emilia et al (1996) recently reported four cases with CR and two cases with PR on CyA in five refractory patients with ITP and one patient with Evans syndrome. Most patients remained drug-dependent but, in contrast to our own observations, side-effects were moderate and transient. The combined CR and PR rate in our group 1 and 2 patients was 55%. The response rate in splenectomy-refractory patients (group 2) was 60%. The majority experienced major toxicity with hypertension, severe muscle pain and headache being the main reasons for discontinuing CyA. Overall 30% of the patients discontinued CyA because of side effects. Only four cases (two in each group) were free of notable side effects.

We conclude that subsequent use of CyA after failure of initial corticosteroid therapy cannot prevent, but only postpone splenectomy in chronic ITP. However, the combination of CyA and low-dose corticosteroids can be useful in a small percentage of patients with refractory ITP, but treatment toxicity is high and the success rate appears not to be greater than any other treatment modality reported to date.

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CHAPTER 5

SERUM THROMBOPOIETIN LEVELS IN RELATION TO DISEASE STATUS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA.

M.C. Kappers-Klunne[1], M. de Haan[1], P.C. Struijk[2], H.H.D.M. van Vliet[1].

[1]Department of Haematology

[2]Department of Obstetrics and Gynaecology, University Hospital Rotterdam, The Netherlands

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SUMMARY

Pre- and post-treatment serum thrombopoietin (TPO) concentration was measured in 35 patients with immune thrombocytopenic purpura. Mean post-treatment levels were significantly lower (p= 0.02) than pre-treatment and not different for treatment modality. No significant correlation between pre- or post-treatment TPO and platelet counts was demonstrable (R= -0.325, P=0.056 and R=-0.227, P=0.190 respectively). In patients with very low platelet counts (≤20 x 10^9/l), pre-treatment serum TPO was significantly higher than in patients with higher counts (p=0.033). The logarithm of the platelet turnover rate, measured in 15 patients, correlated with pre-treatment TPO levels (R = 0.64). These findings suggest a contributory role for TPO in the mechanism of ITP.

INTRODUCTION

Immune thrombocytopenic purpura (ITP) is an immune disorder that is characterized by the presence of anti-platelet autoantibodies and is defined by isolated thrombocytopenia resulting from premature clearance of sensitized platelets through the mononuclear phagocytic system.
Thrombopoietin (TPO) is the primary physiological regulator of megakaryocyte development and of platelet production (Kaushansky et al, 1995). High blood TPO levels have been demonstrated in patients with production-related thrombocytopenia, for example aplastic anemia (Marsh et al 1996, Schrezenmeier et al, 1998). In ITP, TPO levels are only mildly elevated or even within the normal range as is observed in healthy subjects (Kosugi et al,1996; Hou et al, 1998;, Porcelijn et al, 1998). Data regarding TPO levels in relation to disease status, particularly during active disease and after treatment, are scarce (Kosugi et al, 1996; Mukai et al, 1996). We investigated serum TPO levels in ITP patients before and after a treatment episode and correlated pre-treatment serum TPO with platelet turnover rate in a subset of patients.

PATIENTS AND METHODS

Patients.
Thirty-six consecutive patients with ITP presenting at the University Hospital Rotterdam who gave informed consent to take blood samples for the analysis of disease parameters were studied. One patient was only evaluable for pre-treatment serum TPO and platelet kinetic studies.
The criteria for diagnosis included isolated thrombocytopenia, absence of splenomegaly and normal or increased number of bone marrow megakaryocytes. Mean pre-treatment platelet count was 25 x 10^9/l. Bone marrow aspirates were performed in all patients. Mean megakaryocyte number (as determined in at least 6 consecutive fields of vision in a standard light microscope equipped with 10x objective) was 5 per field of vision (range 3-35, normal 2-4). Platelet kinetic studies, performed before the start of treatment, were available in 15 patients. The methods used were as those described previously (International Committee for

Standardization in Haematology, 1988). Briefly, autologous platelets were labeled with [^{111}In]-tropolonate. Blood samples were collected at 1, 2, 3, 4 and 5 h after injection of labelled platelets and subsequently daily until ≤ 10% of the radioactivity of the 1 h sample remained. Platelet recovery was calculated from the total blood radioactivity extrapolated to time zero as a fraction of the injected platelet-bound radioactivity. The mean platelet survival time was calculated according to the multiple hit model (Murphy & Francis, 1971). Platelet turnover rate was calculated using the formula:
platelet turnover (platelets x 10^9/l/day) = blood platelet count/l divided by platelet survival time (days) x recovery (decimal). Mean platelet lifespan in 15 ITP patients was 1.0 day (range 0.03 - 2.8, normal 6 – 10 days). The platelet turnover rate was increased in six patients, within the normal range in seven and decreased in two patients (Fig 1).
Complete remission (CR) was defined as platelets ≥140 x 10^9/l, partial remission (PR) as platelets ≥ 60 and <140 x 10^9/l and non-responder as platelets always < 60 x 10^9/l.
Treatment regimens included corticosteroids (n=7), splenectomy (n=25), high-dose intravenous immunoglobulin (n=2) and interferon α-2b (n=1). CR was attained in 33 treatment episodes, which in 25 cases lasted >2 years (follow-up duration). PR was reached in 1 case and there was 1 non-responder.
Serum samples taken at diagnosis and/or at relapse (pre-treatment sample) as well as after completion of a treatment course (post-treatment sample) were analysed.

Methods.
After clotting and centrifugation, serum samples were stored at -80^o C. Thrombopoietin concentration was measured with a quantitative sandwich enzyme immunoassay technique (Quantikine ^{TM}, R and D systems, Minneapolis, MN, U.S.A.) according to the manufacturer's instructions. Briefly, 50µl of assay diluent and 200µl of recombinant human TPO standards, blank or serum test samples were added to the wells of a microtitre plate, which was precoated with a murine anti-TPO monoclonal antibody and incubated for 3 hours at 4^{o}C. After washing, 200µl of a horseradish peroxidase-conjugated anti-TPO monoclonal antibody was added to each well and the plate incubated for 1 h at 4^{o}C. Another washing procedure and addition of 200 µl substrate solution were followed by incubation at room temperature for 30 minutes. The reaction was stopped by adding 50 µl of 2N sulfuric acid and the optical density determined within 30 min at 450 nm. The minimum detectable TPO concentration was < 15 pg/ml as indicated by the manufacturer. The median serum TPO level in 35 normal blood donors was 104 pg/ml (mean 126 ± 91, range 3 – 532) and 976 pg/ml (mean, 1157 ± 450.8, range 797 - 1800) in four patients with production related thrombocytopenia (severe aplastic anemia in three and chemotherapy-induced bone marrow aplasia in one case). In five patients in whom a second blood sample was taken 1 – 5 months after the first post-treatment sample, TPO concentrations were in the same range as in the first sample.

Statistics.
Standard statistical methods were used. The Wilcoxon signed rank test was applied to assess differences between paired variables. $P< 0.05$ was taken as the level of significance.

RESULTS

Pre- and post- treatment serum TPO concentration was measured in 35 patients. Overall, mean post-treatment serum TPO levels (66.29 pg/ml, SD 36.84) were significantly lower than pre-treatment levels (87.91 pg/ml, SD 60.19, p=0.02) and not different for treatment regimen. However, in eight cases post-treatment serum TPO concentration was higher than pre-treatment and in three cases low pretreatment levels remained unchanged after treatment.
There was no significant correlation between pre- or post-treatment serum TPO concentration and platelet count (R=-0.325, P=0.056 and R=-0.227, P=0.190 respectively). But, in the subgroup of patients presenting with very low platelet counts (≤20 x 10^9/l), pretreatment serum TPO was significantly higher than in patients with higher platelet counts (p=0.033, Fig 2). In 15 patients in whom platelet kinetic studies were performed, a positive correlation between pre-treatment serum TPO levels and the logarithm of the platelet turnover rate was found (R 0.64, p=0.010, Fig 1).

Fig 1. Serum TPO in relation to platelet turnover rate in 15 patients with chronic ITP.

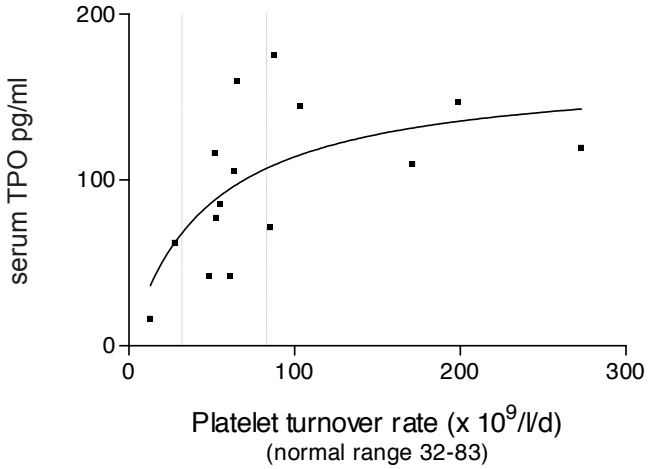
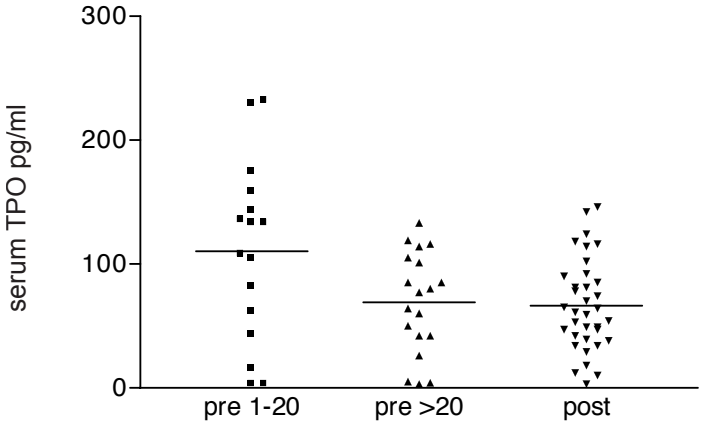


Fig 2. Serum thrombopoietin concentration before (pre) and after (post) treatment in 35 chronic ITP patients. Pre 1-20: patients presenting with platelet counts ranging from 1-20 x 10^9/l; pre >20: patients presenting with > 20 x 10^9/l platelets. Horizontal lines indicate the mean of each group.



DISCUSSION

Pre-treatment serum TPO in our patients always was within the normal range, but showed a wide interindividual variation. This confirms data in the literature indicating that TPO measurement can be important in the diagnostic differentiation of the cause of thrombocytopenia because TPO levels are inversely related to platelet mass in production-related thrombocytopenias, whereas they are normal or only marginally increased in thrombocytopenic states caused by increased destruction (Emmons et al, 1996; Marsh et al, 1996; Mukai et al, 1996; Folman et al, 1997; Schrezenmeier et al, 1998). Remarkably, pretreatment serum TPO levels were significantly higher in patients with very low platelet counts ($\leq 20 \times 10^9$/l) than in those with higher counts, which is consistent with the assumption that binding of plasma TPO by circulating platelets is the main regulatory mechanism of platelet production. In contrast, serum TPO levels correlated with the logarithm of the platelet turnover rate as a measure of platelet production. Generally, it has been assumed that platelet production rate is increased and platelet life span shortened in ITP. However, Ballem et al (1987) discerned a subgroup of ITP patients with normal or even decreased platelet production rate in combination with normal platelet survival. In these cases, relative marrow failure might contribute to the thrombocytopenia. In this regard it is interesting to note that in 11 of the 33 patients who reached CR, an aberrant pattern of low pre-treatment serum TPO was found which remained unchanged or even increased, after treatment. Platelet turnover rate was within the normal range as determined in three of these cases. Although no absolute deficiency of TPO has been demonstrated, relative endogenous TPO deficiency could play a role in the pathophysiology of thrombocytopenia in ITP. Therefore, exogenous TPO, perhaps administered at high pharmacological doses, would be efficacious, particularly in patients with a decreased platelet turnover rate.

In conclusion, serum TPO levels, particularly in combination with other parameters such as platelet turnover rate, might be very indicative for the mechanism of thrombocytopenia in ITP.

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CHAPTER 6

SPLENECTOMY FOR THE TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA

M.C. Kappers-Klunne,[1] P. Wijermans,[2] R. Fijnheer,[3] A.J. Croockewit,[4] B. van der Holt,[5] J.T.M. de Wolf,[6] B. Löwenberg,[1] A. Brand[7]

[1]Department of Hematology, Erasmus MC, Rotterdam
[2]department of internal medicine, HagaZiekenhuis, Den Haag
[3]department of hematology, University Medical Centre Utrecht
[4]department of hematology, University Medical Centre St Radboud, Nijmegen
[5]department of trials and statistics, Erasmus MC, Daniel den Hoed Cancer Centre
[6]department of hematology, University Medical Centre Groningen,
[7]department of hematology and Sanquin Bloodbank South West Region, Leiden University Medical Centre, Leiden

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SUMMARY

Plasma exchange is the treatment of choice for patients with thrombotic thrombocytopenic purpura (TTP) and results in remission in >80% of the cases. Treatment of patients who are refractory to plasma therapy or have relapsing disease is difficult. Splenectomy has been a therapeutic option in these conditions but its value remains controversial.
We report on a series of 33 patients with TTP who were splenectomized either because they were plasma refractory (n=9) or for relapsed disease (n=24). Splenectomy generated prompt and unmaintained remissions in all except five patients, in whom remission was delayed (n=4) or who died with progressive disease (n=1). Four post-operative complications occurred: one pulmonary embolism and three surgical complications. Median follow-up after splenectomy was 109 months (range 28-230 months). The overall post-splenectomy relapse rate was 0.09 relapses/patient-year and the 10-year relapse free survival was 70% (95% CI 50-83%). In the patients with relapsing TTP, relapse rate fell from 0.74 before to 0.10 relapses/patient-year after splenectomy (P <0.00001). Two patients died from first postsplenectomy relapse.
Although these results are based on retrospective data and the relapse rate may spontaneously decrease with time, we conclude that splenectomy when performed during stable disease has an acceptable safety profile and should be considered in cases with plasma refractory or relapsing TTP to reach durable remissions and to reduce or prevent future relapses.

INTRODUCTION

Thrombotic thrombocytopenic purpura is a rare disorder, characterized by the formation of platelet thrombi in the microcirculation. This results in thrombocytopenia, microangiopathic hemolytic anemia and organ dysfunction such as neurologic symptoms and renal insufficiency. The mechanism was elusive until the discovery of a deficient activity of a specific von Willebrand factor-cleaving (vWF-cleaving) protease in patients with TTP (Furlan et al, 1996; Tsai, 1996). Subsequently immunoglobulin G (IgG) inhibitors of vWF-cleaving protease have been detected in the majority of patients with the acquired form of the disease (Furlan et al, 1998a; Tsai et al, 1998). Recently vWF-cleaving protease has been characterized as ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type I repeats), a member of the ADAMTS family of metalloproteases (Fujikawa et al, 2001; Zheng et al 2001). In case of deficient ADAMTS13 activity, highly adhesive ultra-large vWF multimers secreted by endothelial cells cannot be physiologically degradated and may induce platelet clumping in the microcirculation.
Both constitutional and acquired deficiencies of ADAMTS13 have been recognized. When untreated, the disease is almost unvariably fatal (Amorosi & Ultmann, 1966). Since its introduction at the end of the 1970's, plasma exchange has proven to be very effective as the initial treatment for TTP, with response rates of about 80% (Rock et al, 1991; Bell et al, 1991). However, more than one-third of the patients will experience one or more relapses (Onundarson et al, 1992; Shumak et al, 1995; Bandarenko & Brecher, 1998). For patients who

are refractory to plasma exchange and for those with relapsing disease, treatment has been problematic.

The role of the spleen in TTP has been controversial. In the preplasma therapy era, splenectomy was often performed in the acute phase of disease but outcome has generally been dismal in these highly unfavourable situations (Bernard et al, 1969; Kennedy et al, 1980; Bukowski et al, 1981). More recent reports have shown that splenectomy might be effective in plasma refractory cases (Hayward et al, 1994; Winslow & Nelson, 1995; Mant et al, 1999; Rosen et al, 2002) as well as in relapsing TTP (Onundarson et al, 1992; Crowther et al, 1996; Schwartz et al, 2001; Aqui et al, 2003; Zomas et al, 2003) but the results are based on small series of patients. In the present study we report on the value of splenectomy in a series of 33 patients with TTP who underwent splenectomy in the course of their disease.

METHODS

Patients

Patients with acquired TTP, consecutively admitted from January 1982 to May 2002 at six tertiary hematological comprehensive care centres that treat the majority of patients with TTP in the Netherlands, who underwent splenectomy and were followed for >24 months, were included in this retrospective study. Diagnostic criteria for TTP were the presence of thrombocytopenia and microangiopathic hemolytic anemia with at least 1% schistocytes in the peripheral blood smear in the absence of any other identifiable cause (Allford et al, 2003; Vesely et al, 2003; Burns et al, 2004).

Five patients had TTP associated with pregnancy (patients 8, 26, 27, 30, and 32). None of the patients had an associated disease, either at the time of diagnosis or that became apparent during follow-up including human immunodeficiency virus infection. None of them had medication known to be related with TTP or had a positive family history for TTP. Thirteen patients have been the subject of two previous reports (Veltman et al, 1995; Kappers-Klunne et al, 1997) and were included in this study with extended follow-up. Follow-up was complete to the present time or until death in all patients except in two that were lost for follow-up 199 and 108 months respectively, after splenectomy. At the of writing, the majority of the patients were still followed in the out-patient department of the hospital where their splenectomy had been performed. Patients who were discharged from follow-up were contacted by telephone by the treating physician to gather information regarding their general health and relapse status since last noted contact in the medical file. Informed consent was obtained from each patient during one of the recent patient contacts at the outpatient department or by telephone from those who were discharged from follow-up.

Definitions. The day of diagnosis was the day of the first plasma exchange. Refractoriness to plasma exchange: no remission after at least 14 daily plasma exchanges. Remission was defined as a condition with a platelet count >120 x 10^9/l and a serum lactic dehydrogenase (LDH) value of less than 125% of the upper normal level, for > 4 weeks after stopping all treatment. Short lasting exacerbation implied a mild thrombocytopenia not <80 x 10^9/l and serum LDH less than 125% of the upper normal level, absence of neurological symptoms and

reaching remission < 1 week after re-institution of plasma exchange, which then was stopped. Relapse was defined as the recurrence of TTP with platelet count <80 x 10^9/l and LDH more than 125% of the upper normal level following a remission of at least 4 weeks.
Two groups of patients (A and B) were discerned. Group A (nine patients) included patients who were splenectomized because of being refractory to, or remaining dependent on, plasma exchange. Group B (24 patients) comprised patients with relapsing disease who underwent splenectomy in an attempt to prevent relapse. In the majority of the patients splenectomy was performed by the standard open surgical procedure (n= 29) but in four more recently operated cases (patients 6, 24, 32 and 33) the laparoscopic technique was followed. All patients had received pneumococcal vaccine before surgery.

Statistical analysis
The main end point of the study was relapse rate, which was calculated as the number of relapses per patient-year. In group B, the relapse rate after splenectomy was compared with the relapse rate before splenectomy, assuming an exponential survival function for the time to (next) relapse, and the corresponding P-value was calculated using the likelihood ratio statistic. The reported P-value is two-sided; $P < 0.05$ was considered significant. The secondary endpoint was relapse-free survival (RFS) which was calculated from splenectomy until relapse or death, whichever came first. Patients who were still alive at the date of last contact were then censored. RFS was estimated by the Kaplan-Meier method, and a Kaplan-Meier curve was generated to illustrate RFS. All statistical analyses were performed with STATA version 8.2 (StataCorp. 2003; Stata Statistical Software: Release 8. College Station, TX, USA: StataCorp LP).

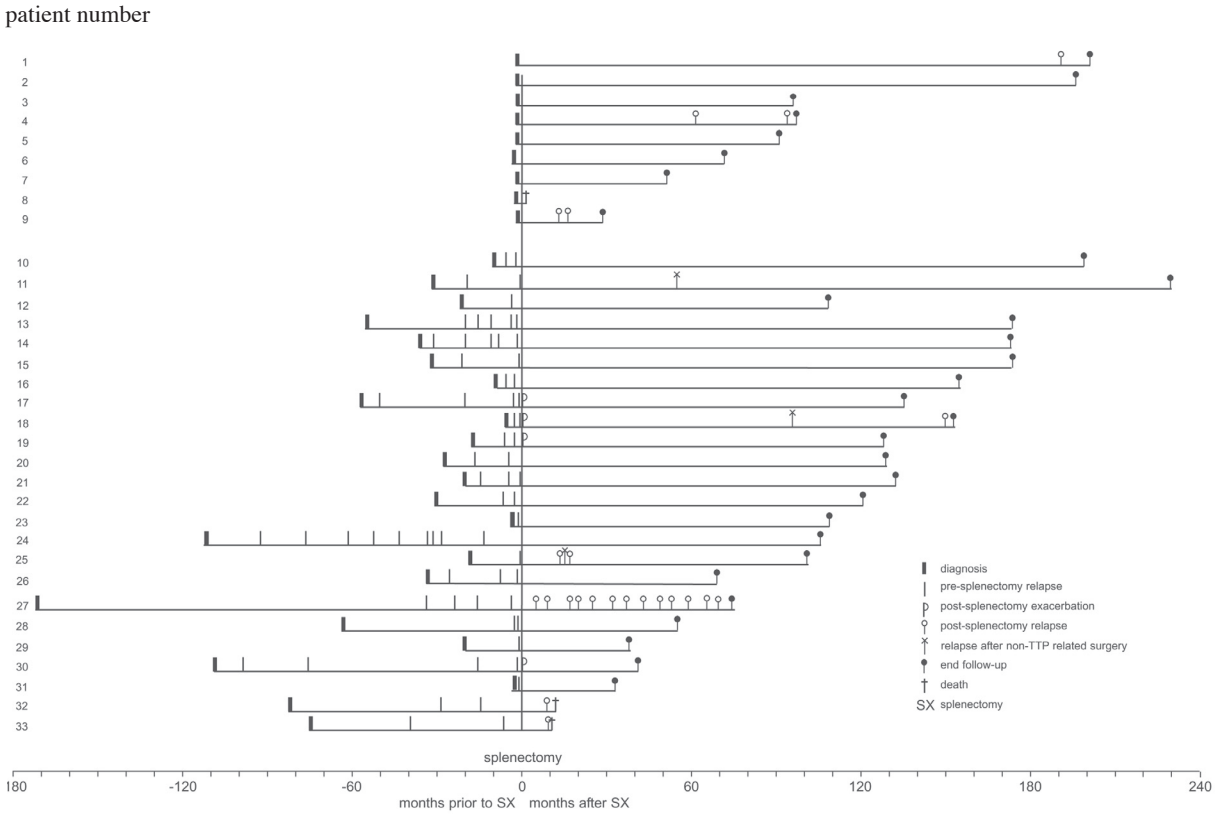
RESULTS

Thirty three patients admitted with the diagnosis TTP had undergone splenectomy during the study period, nine of whom were in group A and 24 in group B (Table I, A and B).

Group A.
Eight patients (patients 1-5, 7-9) in group A had been refractory to plasma exchange (40-50 ml/kg daily) and one patient (patient 6) could not be waned from plasma treatment for almost 3 months. All but two patients (patients 1 and 3) had additionally received prednisone (1-1.5 mg/kg/day), see Table I A. Splenectomy was performed at a median of 24 d (range 14-84 d) after diagnosis. All patients underwent surgery while in the stable phase of disease except patient 8 who had an emergency splenectomy because of rapidly deteriorating disease. This patient died within 24 h after surgery. The cause of death was attributed to ongoing disease as judged by the treating team of physicians. Prompt and durable, unmaintained remissions ensued within 7 – 10 d postoperatively in patients 1-5, 7 and 9. Patient 6 required continued plasma exchange (40 ml/kg every other day) for 2 weeks after splenectomy and subsequent re-institution of immunosuppressive treatment with prednisone 0.25 mg/kg/d in combination with ciclosporin A 3-5 mg/kg/d in a tapering dose for 3 months to reach a sustained and

unmaintained remission. In one patient (patient 7) who used oral contraceptive medication, a bilateral pulmonary embolism occurred postoperatively. After immediate institution of anticoagulant medication recovery was otherwise uneventful.
In group A the relapse rate after splenectomy was estimated at 0.07/patient-years during a median follow-up period of 91 months (range 28-201 months; Fig 1).

Fig 1. Time to splenectomy, duration of remission and number of pre-and postsplenectomy relapses according to year of splenectomy (1982-2002) in patients with plasma refractory (patients 1-9) or with relapsing TTP (patients 10-33).



Group B.
Seven patients in group B were splenectomized in remission (patients 11, 24, 26, 28, 31, 32 and 33) or during stable phase of relapse while on plasma exchange (40-50ml/kg/d). The majority of the patients additionally received prednisone (1-1.5 mg/kg/day). Splenectomy was performed at a median of 31 months (range 4-173 months) after diagnosis and at a median of 1 month (range 0.5-13 months) after onset of the last relapse (Table I B). In two cases (patients 12 and 26) accessory spleens were removed as well. Three patients had postoperative complications, i.e. a wound fistula in patient 15 and a subphrenic abcess in patient 29, which both resolved with conservative measures within 3 weeks. In patient 31, a traumatic lesion of the pancreatic tail occurred during surgery which required a 4-week period of drip-feeding. In all three patients recovery was complete and without long-term sequelae.

TABLE I (A and B).
Characteristics of 33 patients with plasma-refractory or relapsing TTP according to year of splenectomy from 1982-2002
(A) - Group A, patients with *refractory* disease

Patient	age (years) / sex at splenectomy	time from diagnosis to splenectomy (days)	previous treatment	number of post-splenectomy relapses	time from splenectomy to last contact (months)	remarks
1.	36 / F	24	Fprost	1	201	
2.	44 / M	24	FP	0	196	
3.	32 / M	23	F	0	96	
4.	26 / F	21	FPV	2	97	
5.	39 / F	35	FPCr	0	91	
6.	29 / F	84	FPVCy	0	72	remission attained 3 months post-splenectomy after prolonged treatment with P and CsA
7.	18 / F	26	FPCr	0	51	pulmonary embolism 23 days after splenectomy
8.	23 / F	14	FP	na	0.03	died <24 h after splenectomy from progressive disease
9.	61 / F	14	FP	2	28	2nd relapse treated with rituximab followed by sustained remission for 12+ months

F, plasma exchange with fresh frozen plasma; Cr, plasma exchange with cryosupernatant plasma; P, prednisone; V, vincristin; prost, prostacyclin; Cy, cyclofosfamide; CsA, ciclosporin A; na, not applicable

TABLE I (Continued)
(B) - Group B, patients with *relapsing* disease

Patient	age (years) / sex at splenectomy	time from diagnosis to splenectomy (months)	number of relapses pre- versus post-splenectomy		time from splenectomy to last contact (months)	remarks
			pre	post		
10.	34 / M	9	2	0	199	
11.	42 / F	32	2	1	230	relapse 54 months post-splenectomy following surgery
12.	31 / F	22	1	0	108	
13.	37 / M	54	5	0	174	
14.	40 / F	37	5	0	175	
15.	36 / F	33	2	0	174	post-splenectomy woundfistula
16.	28 / F	8	2	0	155	
17.	20 / F	57	4	0	138	short-lasting post-splenectomy exacerbation
18.	44 / F	5	2	2	153	short-lasting post-splenectomy exacerbation; relapse 96 months post-splenectomy following surgery
19.	32 / F	17	2	0	127	short-lasting post-splenectomy exacerbation
20.	29 / F	27	2	0	129	
21.	29 / F	20	3	0	132	
22.	29 / F	30	2	0	121	
23.	52 / F	4	1	0	109	
24.	31 / F	111	9	0	106	
25.	44 / F	17	1	4	102	including 1 relapse 15 months post-splenectomy after surgery
26.	33 / F	33	3	0	70	
27.	24 / F	173	4	13	75	
28.	29 / F	62	2	0	56	
29.	39 / F	20	1	0	38	post-splenectomy subphrenic abscess
30.	21 / F	108	4	0	41	short-lasting post-splenectomy exacerbation
31.	76 / F	4	1	0	33	traumatic lesion of the pancreatic tail during surgery
32.	32 / F	82	2	1	12	died in 1st relapse 12 months after splenectomy
33.	31 / F	74	2	1	9	died in 1st relapse 9 months after splenectomy

TABLE II (A and B). LITERATURE REVIEW*

(A) - Splenectomy in patients with *plasma-refractory* TTP

Author (reference)	number of patients with splenectomy	time from diagnosis to splenectomy in days, mean (range)	number of			follow-up from splenectomy in months, mean (range)
			survivors from splenectomy (%)	patients with post-splenectomy relapse	post-splenectomy relapses	
Thompson et al (1983)	2	18, 28	2 (100)	0		8, 4
Schneider et al (1985)	6	12 (7-27)	6 (100)	0		11 (6-14)
Liu et al (1986)	5	11 (6-17)	5 (100)	1	1	18 (8-36)
Wells et al (1991)	2	21, 26	2 (100)	0		6, 36
Hayward et al (1994)	13	14 (1-26)	11 (85)	0		20 (6-67)
Winslow et al (1995)	6	8 (2-23)	6 (100)	0		21(6-38)
Mant et al (1999)	7	34 (27-52)	6 (86)	0**		29 (18-37)
Delarubia et al (2000)	2	ns	2 (100)	0		11, 26
Wichmann et al (2001)	2	30, 356	2 (100)	0		11, 32
Rosen et al (2002)	9	ns	9 (100)	1	1	13 (1-30)
Aqui et al (2003)	6	ns	6 (100)	0		147 (72-176)†
This study	9	29 (14-84)	8 (89)	3	5 (0.07 / patient-year)	92 (28-201)
Total	69		65 (94)	5	7 (0.03 / patient-year)	30 (1-201)

* series from 1980 reporting > 1 case and number of patients with relapse and/or follow-up from splenectomy;**1 non-responder died 73d post-splenectomy;
†follow-up data available available in 4/6 patients ;ns, not stated.

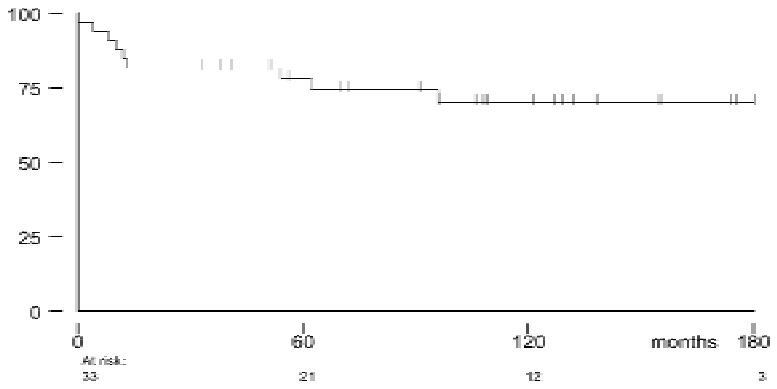
TABLE II. LITERATURE REVIEW* (cont)
(B) - Splenectomy in patients with *relapsing* TTP.

Author (year of publication)	number of patients with splenectomy	time from diagnosis to splenectomy in months mean (range)	number of				follow-up from splen-ectomy in months, mean, (range)
			pre-splenectomy relapses	survivors from splenectomy (%)	patients with post-splenectomy relapse	post-splenectomy relapses	
Liu et al (1986)	2	4, 24	ns	2 (100)	0		7,7
Eldor et al (1992)	2	18, 24	ns	2 (100)	1	1	28, 35
Onundarson et al (1992)	6	6 (0.6-28**)	ns	6 (100)	0		44 (11-68)
Höffkes et al (1995)	2	2, 36	4	2 (100)	0		24, 24
Crowther et al (1996)	6	44 (6-96)	26 (1.18 patient-year)	6 (100)	2	3 (0.13 / patient-year)	45 (10-96)
Delarubia et al (2000)	2	ns	6	2 (100)	1	1	4, 24
Schwartz et al (2001)	7†	68 (1-192)	3 (1-10)‡	7 (100)	0		32 (19-54)
Modic et al (2002)	2	ns	ns	2 (100)	0		5, 13
Essien et al (2003)	2	36, 108	3, 12	2 (100)	0		16, 19
Zomas et al (2003)	5§	ns	>2	5 (100)	0		13‡‡
Kremer Hovinga et al (2004a)	2	8, 12	3 (1.8 / patient-year)	2 (100)	0		44, 91
Aqui et al (2003)	8	ns	29 (1.00 / patient-year)	7 (88)	3	7 (0.33 / patient-year)	31(1-67)
This study	24	43 (4-173)	64 (0.74 / patient-year)	24 (100)	6	22 (0.10 / patient-year)	111 (9-230)
Total	70			69 (99)	13	34 (0.10 / patient-year)	32 (1-230)

* series from 1980 reporting > 1 case and number of patients with relapse and/or follow-up; **including 3 relapses defined as occurring within 1 month of discontinuation of plasma exchange; †2 patients with refractory TTP included; ‡ mean number (range); §1 patient with refractory TTP included; ‡‡median, result not included in total mean follow-up;ns, not stated.

Rapid remissions after splenectomy were attained in 17/21 patients within 3-7 d when all treatment could be stopped. Four patients (17, 18, 19 and 30) had a short-lasting exacerbation of disease in the immediate postoperative period which was managed with a short course of plasma exchange in all cases. In group B, the relapse rate fell significantly from 0.74 relapses/patient-year before splenectomy to 0.10 relapses/patient-year after splenectomy ($P < 0.00001$) during a median follow-up of 115 months (range 9-230 months; Fig 1). In three patients (patients 11, 18 and 25) relapse might have been provoked by surgery, i.e. for perforated duodenal ulcer, inflamed gall bladder and stomach perforation at 54, 96 and 15 months after splenectomy respectively. Two patients (patients 32 and 33) died in first postsplenectomy relapse after 12 and 9 months respectively. The overall 10-year RFS was 70% (95% CI 50-83%; Fig 2)

Fig 2. Kaplan-Meier curve of relapse-free survival from time of splenectomy in 33 patients with TTP. The 10-year relapse-free survival was 70% (95% CI 50-83%).



HISTOPATHOLOGY OF THE SPLEEN

The mean splenic weight was 70-400 g. Histologic examination of the spleen preparations indicated no significant pathology. Subendothelial hyaline deposits, mild extramedullary hematopoiesis and follicular hyperplasia were noted in incidental cases. Microthrombi were detected in two cases (patient 8 and 29).

DISCUSSION

Splenectomy was first used for the treatment of TTP in 1927 (Baehr et al, 1936). The combination of splenectomy and corticosteroids has been applied on an empirical basis for 20 years with reported response rates of about 50% (Bernard et al, 1969; Bukowski et al, 1981;

Schneider et al, 1985). Introduction of plasma therapy by the end of the 1970s significantly improved the prognosis of patients with TTP with remission rates of over 70-80% (Bell et al, 1991; Rock et al, 1991). With the advent of plasma therapy, splenectomy fell into oblivion and was predominantly performed in patients primarily refractory or with progressive disease despite plasma exchange. But under these circumstances splenectomy often was ineffective and associated with a high fatality rate of up to 40 % (Bernard et al, 1969; Rutkow, 1978; Cuttner, 1980). More recently however, splenectomy has been reported to be safer and effective in smaller series of TTP patients with primary refractoriness to plasma exchange or with relapsing disease. In a total of 60 reported cases with refractory disease (Table II A) there were three deaths in the immediate postoperative period, in one case probably because of ongoing disease (Mant et al, 1999) and in the other two cases the cause was not stated (Hayward et al, 1994). Similarly we observed one postoperative death because of progressive disease out of nine patients splenectomized because of non-response to plasma exchange. In 46 patients with relapsing TTP (Table II B) one postoperative death because of ongoing disease was reported (Aqui et al, 2003). By avoiding splenectomy in the active phase of disease we did not observe any deaths. Four major postoperative complications were encountered, i.e. pulmonary embolism in a woman taking oral contraceptives and three surgical complications that occurred in patients who underwent open surgery. After appropriate treatment recovery was complete without long-term sequelae in all cases. Nowadays the laparoscopic approach is preferred in most centres which may further diminish operative complications (Schwartz et al, 2001; Wichmann et al, 2001; Modic et al, 2002; Rosen et al, 2002; Essien et al, 2003).

In all but five surviving patients prompt remissions were attained enabling discontinuation of treatment after splenectomy. Four cases attained a sustained remission after a short lasting exacerbation of disease and in one case remission was delayed and needed a tapering course of plasma exchange in combination with immunosuppressive treatment for a total of three months. Two patients (patients 1 and 3) in group A were only treated with plasma exchange and it remains questionable whether additional treatment with corticosteroids would have avoided splenectomy in these cases. As for the effectiveness of splenectomy in the prevention of relapse in group A patients with refractory disease, five relapses were noted in three patients accounting for a relapse rate of 0.07/patient-year. In a total of 60 patients reported from 1980 in the literature, two relapses were recorded in two patients, corresponding to a relapse rate of 0.02/patient-year (Table II A). In the relapsing cases in group B, the relapse rate in our series decreased from 0.74/patient-year before splenectomy to 0.10/patient-year after splenectomy, equalling a calculated post-splenectomy relapse rate of 0.10/patient-year as reported in 45 patients in the literature since 1980 (Table II B). In one patient with relapsing TTP (patient 27) the frequency of relapse was unaltered after splenectomy. Besides the presence of strong anti-ADAMTS13 inhibitor activity with coexistent absence of ADAMTS13 activity during each relapse (data not shown), no particular cause for the ongoing relapsing disease activity in this patient could be identified.

The positive results of splenectomy observed in this study cannot be attributed with certainty to splenectomy in each case. But, the group A patients were refractory to plasma treatment and the occurrence of a prompt and sustained remission after a new intervention is at least

highly suggestive for a causal role. The same applies for the majority of patients in group B, where the relapse rate was significantly reduced after splenectomy. Nevertheless, spontaneous relapses have been noted in eight patients, even more than 10 years after splenectomy (patients 1 and 18) (Fig 1). As for the cause of these relapses one can only speculate. A better understanding of the mechanisms underlying the therapeutic effect of splenectomy may enable better definition of the indications for surgery.

Histopathology of resected spleens of TTP patients showed widespread occlusive microvascular thrombosis. In addition, a varied pattern of subendothelial hyaline deposits, hemophagocytosis, hemosiderosis, extramedullary hematopoiesis, follicular hyperplasia and periarteriolar concentric fibrosis has been described (Kadri et al, 1975; Asada et al, 1985; Saracco & Farhi, 1990). Dang et al, 1999 found evidence of enhanced microvascular endothelial cell apoptosis in splenic tissue of TTP patients and suggested this was of pathophysiological importance in idiopathic TTP. Arteriolar thrombi were found in two patients in this series, one of whom underwent surgery during active disease (patient 8). The absence of thrombi in the spleens of the majority of our patients might be explained by the fact that splenectomy was performed during clinically stable disease or in remission.

Circulating IgG-autoantibodies with inhibitory activity against ADAMTS13 have been demonstrated in varying percentages (44-94%) of patients with acquired TTP, depending on the diagnostic criteria and assay methods used (Furlan et al, 1998a; Tsai & Lian, 1998; Veyradier et al, 2001; Vesely et al, 2003; Kremer Hovinga et al, 2004a; Peyvandi et al, 2004; Zheng et al, 2004; Böhm et al, 2005). The presence of these inhibiting auto-antibodies argues for an auto-immune nature of the disease. Such auto-immune nature is compatible with the disease course, which can be monophasic in nature, chronic progressive or remitting-relapsing. As the spleen is a major site of antibody production, splenectomy may remove a large reservoir of B lymphocytes producing the pathogenic autoantibodies thereby accounting for the beneficial effect of splenectomy and the prevention of relapses (Furlan et al, 1998b). If the removal of a large reservoir of B cells producing auto-antibodies is indeed an important mechanism of splenectomy, another, non-invasive, means for suppression of B lymphocyte activity might offer an attractive alternative. Monoclonal anti-CD20 antibody (Rituximab) administration has only minor short-term side-effects although long-term consequences are as yet unknown. Rituximab has been given in incidental cases of TTP at different times during the course of disease and often in combination with other drugs. All but three of 23 patients achieved remission but follow-up is still short (Ahmad et al, 2004; Fakhouri et al, 2004; Sallah et al, 2004; Yomtovian et al, 2004; Reddy et al, 2005). Therefore the value of Rituximab in the treatment of TTP is as yet unclear. In the search for parameters that can be used to tailor patient treatment, it is important to know whether antibodies against ADAMTS13 are useful as a surrogate predictor for disease outcome. The first prospective studies on serial measurement of ADAMTS13 activity and its inhibitory antibody are now being reported (Zheng et al, 2004; Böhm et al, 2005, Galbusera et al, 2005). Analysis of these preliminary data indicate that response to plasma exchange may occur independent of the level of ADAMTS13 or inhibitor activity. ADAMTS13 activity and inhibitor levels in eight more recently diagnosed patients in our series (data not shown) are in agreement with those reported in the literature as in six of these, relapses after splenectomy coincided with

ADAMTS13 inhibitor activity and severely deficient (<10%) ADAMTS13 levels. In the two patients who died in first relapse after splenectomy ADAMTS13 levels remained very low and the persistence or recurrence of inhibitor activity despite splenectomy, appeared to be related to the deterioration of disease in these patients. Obviously, the precise value of serial measurement of ADAMTS13 and inhibitory activity for the monitoring of disease activity and as indicators for treatment response has to be established in larger prospective studies.

Our results suggest that splenectomy can produce remissions in plasma-refractory TTP and may diminish the risk for relapse. Notwithstanding these encouraging results, we realise that our analysis has several limitations. First, because of the retrospective nature, we must consider worst-case referral bias. However, such a referral bias would strengthen our conclusion that splenectomy is effective in the studied patient categories. Secondly, the experience of many hematologists is that the frequency of relapse may spontaneously decrease with time which might have influenced the observed reduction in relapse rate after splenectomy in our series. But the course of TTP is unpredictable and multiple relapses occurred even 5 years or more after diagnosis in six of our patients (patients 24, 27, 28, 30, 32 and 33) before splenectomy was performed. Third, relapses may still follow in patients with the shorter follow-up. Finally, personal preference of the members of the treating team of physicians for a specific type of treatment cannot be completely ruled out.

Despite these limitations, our findings in a large, well-documented group of patients followed for a prolonged period of time, confirm and extend the potential beneficial effect of splenectomy in both plasma refractory as in relapsing TTP as previously suggested in smaller case series with much shorter and often incomplete follow-up.

We conclude that splenectomy, when performed in stable disease phase and preferably by the laparoscopic technique, is an acceptably safe procedure which might be applied in cases with a protracted disease course after failure of or dependency on plasma therapy. Splenectomy may also be seriously considered in patients with relapsing TTP to prevent, or at least reduce the risk of, future relapses. It remains to be established whether measurement of ADAMTS13 and inhibitory antibody levels will improve selection of patients for splenectomy or for a less invasive treatment as with the newly introduced specific anti-B cell monoclonal antibody therapy particularly with its apparent minimal side-effects.

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CHAPTER 7

SUMMARY AND DISCUSSION

SUMMARY AND DISCUSSION.

The mechanisms of immune thrombocytopenias have for a long time been obscure. The different types of thrombocytopenia directly or indirectly relate to immune dysregulation and any of these may cause life threatening bleeding problems. Treatment development in these situations has been largely empirical. In the past few years progress has been made in understanding the pathophysiological mechanism, diagnosis and treatment of thrombocytopenias. The studies described in this thesis deal with these issues.

Heparin-induced thrombocytopenia.

In **chapter 2** we describe a peculiar form of drug-induced immune thrombocytopenia in which a thrombotic risk exceeds the risk of bleeding due to a low platelet count. Since, at the time of designing our study there were no established criteria for the diagnosis of heparin-induced thrombocytopenia and/or thrombosis (HITT), we first set out to define HITT. Nowadays it is generally agreed that a progressive reduction of the absolute platelet count to less than the lower limit of normal (usually a platelet count threshold of 150 x 10^9/l) in combination with positive testing for HITT antibodies are diagnostic of HITT (1). However, patients with a substantial drop of the platelet count but with a nadir that stays above 150 x 10^9/l, may also have HITT. On the other hand, not all patients who make HITT antibodies acquire HITT. With the aim of formulating a specific set of criteria for establishing HITT, we and others considered and applied a relative fall in platelet count of >50% (or >30% in case of concomitant thrombosis) in combination with positive testing for HITT antibodies. A few years later, Warkentin et al confirmed the usefulness of a proportional platelet count decrement in a systematic analysis of a large group of postoperative orthopedic patients (2).

Initial studies on the incidence of HITT reported highly variable percentages ranging from 0-30% (3, 4). These studies were based on relatively small series of patients with different definitions used. Subsequently more information about the incidence of HITT has become available. A variety of factors influence the probability of the development of HITT (5). Surgical patients appear to be at highest risk. Bovine-derived heparin is more likely to induce HITT than porcine-derived preparations. Furthermore, the higher, therapeutic, dose levels of heparin are more often associated with HITT than the lower prophylactic doses (6). Most reports on the incidence of HITT concern patients following surgery.
Applying the diagnostic criteria of a relative decline of platelet numbers and the presence of HITT serum antibodies, we prospectively evaluated the incidence of HITT and HITT antibodies in non-surgical patients who were treated with a therapeutic dose of porcine unfractionated heparin (UFH).

Incidence of HITT.

The incidence of 0.3% of HITT as observed by us in medical patients is less than the incidence rates generally seen in orthopedic and cardiac surgery patients on UFH prophylaxis (see table).

The patients in our series received therapeutic doses of UFH. The fact that we found a low incidence of HITT of only 0.3% is remarkable since a therapeutic dose of UFH has been reported to be associated with higher frequencies of HITT (3, 17, 20). The different incidence rates of HITT among surgical and non-surgical patients may relate to the effect of the surgery itself. This effect may be due to perioperative platelet activation with resulting PF4 release or postoperative thrombocytosis as observed in orthopedic surgery patients.
The incidence of HITT of 0.3% that we noted is comparable with the incidences reported by others in medical patients on prophylactic or therapeutic UFH (see table). The relatively low incidence of HITT in our study may also relate to the duration of UFH treatment which was comparatively short (mean 8.3 days). The latter time interval of heparin exposure may be too short for developing HITT, although this period may be sufficiently long for developing HITT antibodies. In the study of Girolami, the 5 cases of HITT among 598 medical patients (0.8%) all occurred in the group on UFH prophylaxis, with HITT becoming apparent after days 8 to 22 following the start of treatment (17).

As the majority of the patients (67%) in our study group had had a previous exposure to UFH, one would have expected an increased incidence of HITT in our study population. In typical HITT thrombocytopenia occurs about one week after the start of heparin therapy. However, in patients previously treated with heparin, a more rapid fall in the platelet count has been observed, even within several hours. It has also been suggested that an early onset of thrombocytopenia in HITT depends on the interval following the last heparin administration, i.e. beyond or within the previous three months (21, 22). This time period equals the period that HITT antibodies are detectable in the circulation. It is now widely accepted that persistently circulating HITT antibodies can provoke acute HITT on reexposure (21,22). It has also been noted that HITT antibodies do not always recur with subsequent heparin therapy (21, 22).
In our patients with prior administration of UFH, the rechallenge of UFH had taken place at an interval of greater than 3 months (mean time interval 52 months) and none of the preexposed patients had circulating HITT antibodies at the start of UFH. We assume that this may explain the comparatively low incidence of HITT in our preexposed patients.

On the basis of our study and the recent data of others, we conclude that the incidence of HITT in non-surgical patients is generally low (see table). Since UFH is still commonly used and because of the broad use of low molecular weight heparins, HITT will remain a common condition in clinical practice. An active awareness of a possible diagnosis of HITT in patients on heparin therapy who develop thrombocytopenia, is therefore indicated.

TABLE. SELECTED STUDIES ON THE INCIDENCE OF HITT AND HITT ANTIBODIES IN SURGICAL AND NON-SURGICAL PATIENTS.

Author - year of publication (reference)	number of patients	patient category	therapy - UFH and/or LMWH	prophylactic (P) / therapeutic (T)	treatment duration (days, mean)	incidence of HITT (% of total patient number)		incidence of IgG-HITT antibodies (antigen assay) (% of total patient number)	
						UFH	LMWH	UFH	LMWH
Warkentin - 1995 (7)	665	orthopedic surgery	UFH	P	10	2.7	0.0	7.8	2.2
Trossaert - 1998 (8)	51	cardiac surgery	UFH	P	8.9	0.0		27.0	
Warkentin - 2000 (9)	100	cardiac surgery	UFH	P	5.1	1.0		50.0	
Pouplard - 2002 (10)	633	cardiac surgery	UFH and LMWH *	P and T	≥ 10	3.4	0.3	38	
Greinacher - 2005 (11)	502	orthopedic surgery	UFH and LMWH	P	16	2.6	0.0	23.5 #	8.3
Martel - 2005 (12)	1223 (meta-analysis)	mainly orthopedic surgery (4/5 studies)	UFH and LMWH	P		2.6	0.2		
Schmitt - 1993 (4)	pooled analysis of 18 prospective studies	medical	UFH	T		1.1		n.s.	
Boon - 1996 (13)	261	chronic hemodialysis	UFH and LMWH	P	36.8 months (UFH) 45 months (LMWH)	0.0	0.0	2	0.7
Kappers - 1997	356	medical	UFH	T	8.3	0.3		2.5	
Lindhoff - 2002 (14)	1137	DVT treatment	UFH and LMWH	T	nv	0.5	0.5	9.1¥	2.8
O'Shea - 2002 (15)	81	chronic hemodialysis	UFH	P	33.7 months	0.0		1.2	
Palomo - 2005 (16)	207	chronic hemodialysis	UFH	P	35 months	0.0		29.7	
Girolami - 2003 (17)	598	general medical	UFH	P and T	14 (median/prophylaxis) 10 (median/therapeutic)	0.8 **		n.s.	
Prandoni - 2005 (18)	1754	general medical	LMWH	P and T	8.0 (median) §		0.8	n.s.	
Pohl - 2005 (19)	311	neurological	UFH and LMWH	P and T ¶	16.6 (for UFH) 12.6 (for LMWH)	2.5	0.0	20.5	1.8

UFH denotes unfractionated heparin, LMWH denotes low-molecular-weight-heparin;* in 263 patients UFH during surgery and post-operatively continued until at least day 10; ¥ 9.1% on day 5-7 and 20.7% on day 21 of UFH; # measured on day 16; ** n.s. not stated. all in the prophylactic group; § 40% up to 7 days, 40% up to 14 days, 8% up to 21 days, 11% up to 30 days; ¶ UFH prophylactic and therapeutic dose, LMWH prophylaxis only.

Incidence of heparin-dependent antibodies.
Heparin-dependent (HITT) antibodies are detected either with an antigen assay which measures the binding of antibodies to immobilized platelet factor 4-heparin (PF4/hep) complexes (e.g. PF4/hep Elisa) or with a platelet activation assay that measures platelet activation through IgG antibodies (e.g. HIPA test) (23, 24). Different immunoglobulin classes of antibodies have been detected but it seems that patients with a high titer of IgG antibodies are at greatest risk of developing HITT (1).
Few studies have sorted out the incidence of HITT antibodies in non-surgical patients (see table). The incidence of IgG-HITT antibodies of 2.5% as observed by us is low as compared to the incidence values reported by others. Lindhoff-Last et al noted an incidence of IgG-HITT antibodies of 9.1% on days 5-7 after the start of heparin treatment. This value rose to 20.7% on day 21 of UFH therapy in patients treated for deep vein thrombosis (14). The observed increased incidence of HITT antibodies during prolonged treatment provides an argument for the duration of UFH therapy as a prognostic factor for the development of HITT antibodies. A high incidence of HITT antibodies (20.5%) has also been reported in neurological patients on UFH (19).
Remarkably, repeated exposure to UFH for a prolonged period of time, as occurs in patients on chronic hemodialysis, did not clearly enhance the frequency of HITT antibodies. IgG PF4/hep Elisa antibodies were demonstrable in no more than 2% of our 128 cases (13). This low incidence may perhaps be due to impairment of the immune response in the hemodialysis patient population (25).

The role of IgM PF4/hep Elisa antibodies in the development of HITT has not been widely studied and remains controversial (26, 27, 28, 29, 30). We found isolated IgM antibodies in 7 (2%) of 358 patients studied. None of these patients acquired HITT. Amiral et al reported isolated IgM and/or IgA HITT antibodies in 12 (32%) of 38 patients with HITT (26). They hypothesized platelet activation to take place through direct binding of IgM or IgA antibodies to PF4 on the platelet surface. Alternatively, platelet activation might be mediated indirectly either through neutrophils and monocytes, which possess receptors for IgM and IgA, or through lymphocytes which expose FcμR receptors for IgM. In case of IgM antibodies, platelet activation might also be induced by complement fixation. Finally, platelet activating antibodies against other chemokines such as neutrophil-activating peptide-2 or interleukin-8 may be involved (26, 31, 32).
Altogether, the pathogenicity of IgM and IgA antibodies remains poorly defined and has to be clarified in future studies.

As regards the clinical significance of circulating HITT antibodies without HITT, one has to realize that systematic serologic studies addressing the question of the sensitivity and specificity of antibody tests generally have rarely been conducted. These studies whenever done, have been restricted to patients clinically suspected of HITT or specific patient populations and therefore did not yield general estimates. For instance, clinically insignificant antibodies are often detected in patients who had received heparin up to 100 days earlier (33). Hence, the diagnostic specificity of a positive antibody test for HITT is low. On the other

hand, a negative test result usually rules out HITT, i.e. the negative predictive value is high (34).

HITT remains a clinical diagnosis. The demonstration of HITT antibodies confirms a clinical suspicion of HITT. The clinical significance of circulating HITT antibodies without signs of HITT remains as yet unclear.

Thrombocytopenia and platelet alloimmunization.

Patients with congenital or acquired thrombocytopenia may need therapeutic or prophylactic platelet transfusions. The universal introduction of leukocyte depletion of platelet concentrates has drastically diminished primary platelet alloimmunization to less than 5%. However, patients with a transfusion history of non-leukodepleted blood products or with previous pregnancies, remain at considerably greater risk of secondary immunization. Alloimmunization to random donor platelets is in the majority of cases caused by HLA antibodies. To obtain adequate transfusion results, alloimmunized patients can be transfused with HLA matched platelets. Unfortunately however, HLA matched transfusions are not always successful in these conditions.

In chapter 3 the results are described of a retrospective study on the predictive value of HLA antibody testing for the outcome of the first HLA matched platelet transfusion in patients refractory to random donor platelet transfusions, who always had received leukodepleted blood products in case of transfusion. Seventytwo patients received a total of 560 HLA matched platelet transfusions. HLA antibodies were determined with a lymphocyte immunofluorescence test (LIFT) or a monoclonal antibody-specific immobilization of platelet antigens assay (MAIPA). Successful transfusion outcome as assessed at one hour (recovery $\geq$20%) and sixteen hours (recovery $\geq$10%) after an HLA matched platelet transfusion was noted in 87% and 80% of patients with HLA antibodies who had not responded to previous random donor platelet transfusions.

The fact that 13% of the patients with HLA antibodies did not respond to the first HLA matched transfusion might suggest a possible role of non-immune factors in these cases. Non-immunological determinants of unsuccessful transfusions that were evaluated in our study were gender, diagnosis and therapy of hematological disease. None of these parameters were shown to compromise transfusion outcome. Incidentally in these instances, the transfusion failure may have resulted from the simultaneous presence of platelet specific antibodies (see also introduction). Another possible explanation for the unsatisfactory response to HLA matched platelets in HLA antibody positive (sensibilized) patients, might have been the use of a not perfectly matched platelet donor in the absence of a fully HLA matched donor. In these cases, the HLA antibodies might still have played a negative role following transfusion. Indeed, a significantly better recovery after one hour was found when a compatible HLA match was present between the donor and recipient than in case of cross-reactive mismatches.

Considering the fact that some response to HLA matched platelets was noted in the majority of HLA negative patients refractory to random donor platelet transfusions, it is too early to

conclude that HLA testing can be abrogated. Before making a definite decision, these results have to be confirmed in a prospective setting in which the following conditions are fulfilled: a) the sensitization status of the patient has to be definitely established by repeated testing for HLA antibodies to avoid a false negative test outcome, e.g., due to adsorption of antibodies to recently administered donor platelets; b) exclusion of non-immunological parameters such as medication, fever and splenomegaly, in addition to the parameters analyzed in our study; c) exclusion of platelet specific antibodies; d) in the absence of the above, a rechallenge with random donor platelets.

We found that almost 90% of the patients with a positive HLA test can be successfully transfused with an HLA matched platelet concentrate. In HLA antibody positive patients without an adequate recovery after an HLA matched platelet transfusion, a search for non-immunological factors is recommended. In the absence of these, a search for platelet specific antibodies should be considered. The relatively successful outcome of HLA-matched platelet transfusions in random donor refractory, HLA antibody negative patients deserves further prospective study.

Thrombocytopenia caused by platelet autoimmunization.

Fcγ-receptor-mediated clearance of autoantibody-sensitized platelets in the mononuclear phagocytic system, particularly in the spleen, is a primary cause of thrombocytopenia in immune thrombocytopenic purpura (ITP). In the past, treatment has aimed at the suppression of autoantibody production mainly by eradicating the auto-reactive B lymphocyte population. Therefore, immunosuppression has been the cornerstone of treatment in ITP. The results of first-line treatment with corticosteroids is often disappointing with reported success rates of 15-60%. In these cases splenectomy is often performed with the purpose of removing a site of antibody synthesis and platelet clearance. Splenectomy may result in remissions in approximately 70% of the cases (35). We applied an intensive immunosuppressive drug combination of prednisone and cyclosporin A (CyA), given either before (group 1, n=10) or after splenectomy (group 2, n=10), in patients with corticosteroid-refractory ITP **(chapter 4)**. The overall response rate was 55%, i.e. 50% in group 1 and 60% in group 2 patients. Nine of the 10 patients in group 1 proceeded to a splenectomy after a mean of 18 months from the start of CyA. The reasons for splenectomy were, relapse of disease, insufficient response to CyA or intolerance of CyA. Overall, one-third of the patients discontinued CyA because of side-effects.

Obviously, these numbers are too small to allow for firm conclusions. Nevertheless, these experiences would suggest that CyA treatment cannot preclude splenectomy in the majority of corticosteroid refractory patients.

Assuming that the induction of B cell depletion could interfere positively with the production of pathologic antibodies, recently anti-CD20 monoclonal antibody therapy has been introduced as a treatment for ITP. Most experience has been gained with Rituximab which is a human-mouse chimeric monoclonal antibody specific for the CD20 antigen, present on the surface of B-lymphocytes. Preliminary reports show success rates of around 50% (36). The

value of Rituximab in the treatment of ITP is currently the subject of a collaborative study by HOVON (Stichting Hemato-Oncologie voor Volwassenen Nederland).
CyA may be useful in some patients with corticosteroid-, anti-CD20 or splenectomy-refractory ITP.

The role of thrombopoietin in autoimmune thrombocytopenia.
As outlined in the introduction, the mechanism of ITP is still incompletely understood. Autoantibody-mediated platelet destruction has for a long time been regarded as the sole cause of ITP. However, already in the 1980s, autologous platelet kinetic studies had suggested reduced or at best normal platelet production rates in ITP. In the subsequent years, others have brought up additional circumstantial evidence for existing suppression of megakaryo- and thrombopoiesis (see introduction).
We studied two aspects of regulation of thrombopoiesis in 35 patients with ITP (**chapter 5**).
First, serum thrombopoietin (TPO) levels were measured before and after a treatment episode in all patients. Treatment had been successful in 33 of 35 episodes studied. One patient had been a non-responder and the other was a partial responder (platelet counts > 60 x 10^9/l). Pretreatment serum TPO was always within the normal range which is in accordance with literature data (37, 38). Remarkably, severely thrombocytopenic patients (platelet count ≤20 x 10^9/l) showed significantly (p=0.033) higher pretreatment TPO levels than those with less severe thrombocytopenia (platelet counts >20 x 10^9/l). This means that TPO levels inversely correlated with platelet counts, a pattern which is consistent with the assumption of impaired regulation of megakaryopoiesis in ITP.

Secondly, the platelet turnover rate, as a measure for platelet production, was determined with ^{111}In-labelled autologous platelets in a subset of 15 patients. Subsequently, platelet turnover rates were correlated with pretreatment TPO levels. We found a positive correlation (R=0.64) between pretreatment serum TPO concentrations and the logarithm of the platelet turnover rate. In addition to the results of serum TPO measurements, this finding might also suggest impaired regulation of megakaryo- and thrombopoiesis in ITP because of a (relative) shortage of TPO.
Shortly after our publication, another group reported the results of serum thrombopoietin concentrations in a larger cohort of thrombocytopenic patients grouped according to their platelet lifespan, platelet turnover and production rate (39). The results of the latter study are informative as regards the mechanism of thrombocytopenias in general but do not allow for definitive distinction between the different forms of thrombocytopenia except for those with production-related thrombocytopenias. However, the interpretation of the results in different patient categories and in different studies, is hampered by the as yet unexplained wide variation of TPO concentrations that are observed in every patient category as well as in controls.

The data obtained in our study suggest that an inadequate response of TPO may be important in the mechanism of ITP. Platelet kinetic studies in combination with measurement of blood TPO levels in patients presenting with a diagnosis of ITP, may provide a better insight into

the heterogeneity of ITP and may in the future allow for optimizing treatment regimens. Preliminary encouraging results of recently started treatment studies with modified thrombopoietin support the notion of inappropriate TPO levels in ITP (40).

Thrombocytopenia caused by platelet consumption due to an underlying immune disturbance.

Thrombocytopenia in TTP is the result of platelet clumping in the microcirculation due to the persistent circulation of ultra-large vWF (ULVWF) multimers. These hemostatically very active ULVWF multimers are under normal circumstances cleaved by a specific protease (ADAMTS13) which is inhibited by IgG autoantibodies in patients with the acquired form of TTP. Plasma exchange, usually in combination with corticosteroids, is the standard first-line treatment for TTP, resulting in remissions in 70-80% of the cases (41, 42). Treatment of patients with plasma refractory TTP and those with relapsing disease has been notoriously difficult. Splenectomy has been applied on an empirical basis but its mechanism has remained uncertain.

To gain insight into disease outcome in relation to the various treatment modalities that were applied, we first conducted a nationwide inventory in patients admitted to one of 13 hematological centers in the Netherlands between 1979-1992 (43). A total of 65 evaluable patients with newly diagnosed TTP were identified. Ninety-five percent of the patients were treated with fresh frozen plasma. Seventy-four percent (additionally) received corticosteroids. All 52 patients (80%) who survived the first four weeks after admission reached complete remission. The 5-year survival rate was 77% (95% CI:66-87) and the 5-year relapse-free survival rate was 38% (95% CI: 25-52, see figure). These results are in accordance with data in the literature (41, 42).

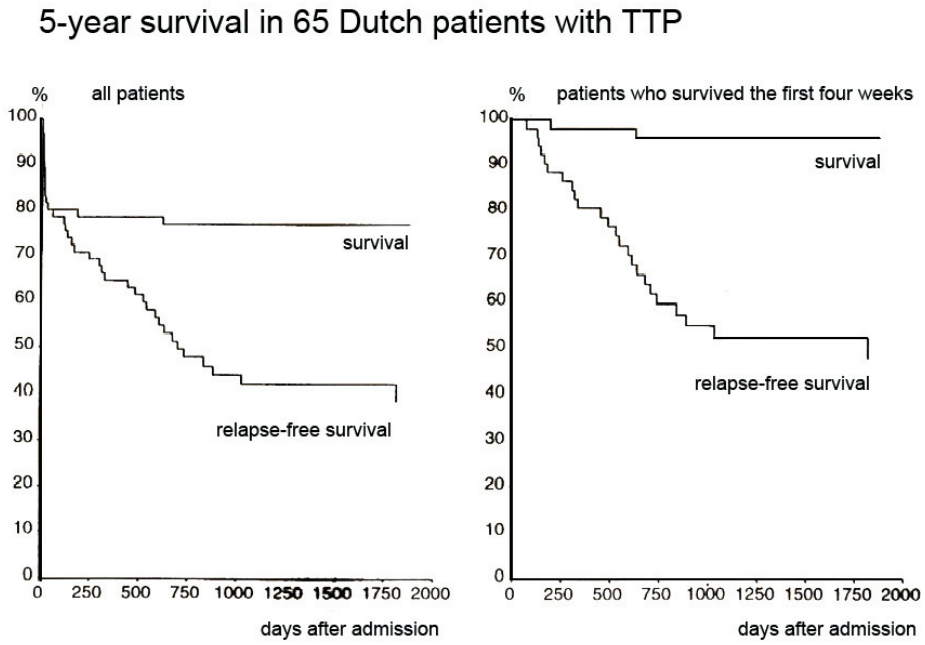
This group of 65 patients included twelve patients with relapsing TTP who had had a splenectomy. The splenectomy had resulted in a durable remission in all cases. We recently extended the experience with splenectomy in an accumulated series of 33 patients with acquired TTP who had been admitted to 6 tertiairy hematological comprehensive care centers in the Netherlands. The results of this analysis are described in **chapter 6**.

Nine patients had been splenectomized for plasma-refractory TTP and 24 for relapsing TTP. Splenectomy had been successful in leading to clinical remissions in all but one of these patients. During a mean follow-up of 69 months (range 33-199 months), stable clinical remissions were noted in 23 patients, i.e. 5 in the refractory group and 18 in the group with relapsing TTP. Nine patients experienced one or more relapses. The overall postsplenectomy relapse rate was 0.09 relapses/patient-year. The 10-year relapse-free survival was 70% (95% CI 50-83%). In the patients with relapsing TTP, the relapse rate fell significantly after splenectomy, i.e. from 0.74 relapses/patient to 0.10 ($P<0.0001$).

It is noteworthy that splenectomy was therapeutically active and relatively safe when performed during stable disease or in remission. Splenectomy may be considered in cases of plasma refractory or relapsing TTP. A better understanding of the role of the spleen in TTP would be desirable to enable a better selection of patients for splenectomy. It remains to be

seen whether the non-invasive treatment modalities such as B-cell directed immunotherapy with anti-CD20 monoclonal antibodies may obviate the need for splenectomy.

Figure*



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CHAPTER 8

SAMENVATTING

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Bloedplaatjes (trombocyten) spelen een belangrijke rol bij het handhaven van de normale bloedstolling. Indien het bloedplaatjesaantal verlaagd is (trombocytopenie) bestaat er een verhoogde kans op bloeding.
Belangrijke oorzaken voor een verlaagd bloedplaatjesaantal zijn: gestoorde aanmaak van de bloedplaatjes in het beenmerg, toegenomen destructie of consumptie van bloedplaatjes, abnormale distributie (miltvergroting) en verlies van bloedplaatjes bij massaal bloedverlies. Als het bloedplaatjesaantal sterk verlaagd is, bestaat er een vergrote kans op ernstige en soms levensbedreigende bloedingen. Op dergelijke momenten is direct therapeutisch ingrijpen vereist. Dit kan betekenen dat een verdacht medicament terstond gestaakt moet worden, maar kan bijvoorbeeld ook aanleiding zijn voor toediening van bloedplaatjes. Als een immunologische stoornis de oorzaak is van het lage bloedplaatjesaantal, zijn dergelijke maatregelen vaak niet effectief en kan de behandeling problematisch zijn.

Wij onderzochten 3 verschillende situaties van trombocytopenie op basis van een immunologische oorzaak.
Ten eerste, trombocytopenie ten gevolge van de vorming van antistoffen gericht tegen de eigen bloedplaatjes (autoantistoffen). Deze trombocytopenie wordt autoimmuun trombocytopenie genoemd. Twee vormen van autoimmuun trombocytopenie werden onderzocht, namelijk trombocytopenie veroorzaakt door het gebruik van het ontstollingsmiddel heparine en trombocytopenie bij patiënten met de ziekte ITP (immuun trombocytopenische purpura).
Ten tweede, trombocytopenie bij patiënten die antistoffen hebben gevormd tegen donorbloedplaatjes (alloantistoffen).
Ten derde, een vorm van trombocytopenie die ontstaan is door consumptie van bloedplaatjes als gevolg van een onderliggende immuun stoornis die optreedt bij patiënten met de ziekte TTP (trombotische trombocytopenische purpura).

Heparine-geinduceerde trombocytopenie en trombose (HITT).
Toediening van heparine kan gepaard gaan met een bijzondere vorm van trombocytopenie waarbij de kans op trombose groter is dan de te verwachten kans op bloeding ten gevolge van het lage bloedplaatjesaantal. Er zijn 2 verschillende vormen van (HITT), een immunologische vorm en een niet-immunologische vorm.
Trombocytopenie is een bijwerking van veel medicamenten. Daar patiënten vaak diverse middelen tegelijk gebruiken kan het moeilijk zijn vast te stellen welk medicament de trombocytopenie heeft veroorzaakt.
Bij de start van onze studie was er geen goed inzicht in het voorkomen van HITT. De in de literatuur gerapporteerde incidentie varieerde sterk en was gebaseerd op relatief kleine studies waarin verschillende definities werden gehanteerd. **Hoofdstuk 2** beschrijft de resultaten van een evaluatie naar de incidentie van de immunologische vorm van HITT bij patiënten die waren opgenomen op de afdelingen cardiologie en neurologie van ons ziekenhuis.

De patiënten werden behandeld met een hogere, therapeutische dosis van ongefractioneerde varkens heparine (UFH).
Ook waren er ten tijde van het ontwerpen van de studie geen algemeen geaccepteerde criteria voor de diagnose HITT. Daarom werden eerst duidelijke criteria opgesteld. Hierbij werd een relatieve daling van het bloedplaatjesaantal gehanteerd, in plaats van een absolute daling tot onder de normaalwaarde, in combinatie met de aanwezigheid van HITT antistoffen in het bloed van de patiënt.

De door ons gevonden incidentie van 0.3% is laag en is vergelijkbaar met de incidentie die door anderen is gevonden bij niet-chirurgische patiënten (zie tabel bij hoofdstuk 7). Deze incidentie van 0.3% is lager dan de incidenties die gevonden worden bij postoperatieve patiënten die een lagere (profylactische) dosis UFH kregen toegediend ter voorkoming van trombose (zie tabel bij hoofdstuk 7). De gevonden lage incidentie van HITT zou mogelijk beïnvloed kunnen zijn door de relatief korte duur van de behandeling met UFH (gemiddeld 8.3 dagen), hoewel deze periode lang genoeg is voor de vorming van HITT antistoffen. Zo traden in de studie van Girolami, de 5 gevallen van HITT (0.8%) op tussen de 8ste en 22ste dag na aanvang van de behandeling met UFH (1).

Gezien het feit dat tweederde van onze patiënten eerder was behandeld met UFH, zou een hogere incidentie van, acuut opgetreden, HITT te verwachten zijn geweest. Echter, het is inmiddels bekend dat acute HITT wordt geluxeerd door HITT antistoffen die in de circulatie aanwezig zijn op het moment van hernieuwde UFH toediening. Deze antistoffen zijn in totaal ongeveer 3 maanden na de eerste toediening aantoonbaar. De eerdere behandeling van onze patiënten met UFH vond plaats langer dan 3 maanden tevoren. Bovendien waren er bij geen van deze patiënten HITT antistoffen aantoonbaar op het moment dat zij opnieuw UFH kregen toegediend.

Hoewel er verschillende immunoglobuline klassen van HITT antistoffen zijn aangetoond, blijken patienten met IgG-HITT antistoffen de grootste kans te hebben op het krijgen van HITT.
Met behulp van de plaatjes factor 4/ heparine (PF4/hep) Elisa test waren bij 2.5% van onze patiënten IgG-HITT antistoffen aantoonbaar zonder dat zij klinische verschijnselen van HITT vertoonden. Anderen vonden een hoger percentage IgG-HITT antistoffen, dat verder steeg bij langere voortzetting van UFH (zie tabel bij hoofdstuk 7) (2). Dit is een aanwijzing dat de duur van de behandeling met UFH een voorspellende factor kan zijn voor de vorming van HITT antistoffen.
Bij de interpretatie van deze bevindingen dient men zich te realiseren dat HITT een klinische diagnose is. Het aantonen van HITT antistoffen bevestigt een klinische verdenking van HITT. De klinische betekenis van de aanwezigheid van circulerende HITT antistoffen zonder de klinische verschijnselen van HITT, is vooralsnog onduidelijk.

Trombocytopenie en bloedplaatjes alloimmunisatie.
Transfusie van bloedplaatjes is een onderdeel van de ondersteunende behandeling van patiënten met aangeboren en verkregen vormen van trombocytopenie. Bloedplaatjestransfusie is vooral belangrijk voor patiënten met acute leukemie en andere kwaadaardige aandoeningen die behandeld worden met chemotherapie. Echter, in het verleden werd een groot deel van de patiënten refractair voor transfusie met bloedplaatjes afkomstig van willekeurige donoren. Gelukkig is na de recente invoering van universele leucocytendepletie van bloedproducten, het percentage patiënten dat niet meer reageert op trombocyten van willekeurige donoren drastisch gedaald tot onder de vijf procent. Patiënten die in het verleden niet-leucocyten gedepleteerde bloedproducten ontvingen of patiënten die zwanger geweest zijn, blijven echter een groot risico lopen refractair te worden voor transfusie met bloedplaatjes van willekeurige donoren.
Immunisatie door HLA-antistoffen is de belangrijkste oorzaak voor refractair worden voor bloedplaatjestransfusie van willekeurige donoren. Indien dit het geval is kunnen patiënten getransfundeerd worden met passende, HLA-getypeerde, bloedplaatjes. Het is daarom voor de clinicus van belang geïnformeerd te zijn over de aanwezigheid van HLA antistoffen bij de ontvanger. In **hoofdstuk 3** worden de resultaten beschreven van een studie naar de voorspellende waarde van het testen op de aanwezigheid van HLA antistoffen voor de uitkomst van de eerste transfusie met passende, HLA-getypeerde bloedplaatjes bij 72 patiënten die refractair waren voor bloedplaatjes van willekeurige donoren en die altijd leucocyten-gedepleteerde bloedproducten hadden ontvangen in geval van transfusie. HLA antistoffen werden aangetoond met 2 verschillende testen, LIFT en MAIPA. Een succesvolle opbrengst, gemeten een uur na toediening van een HLA-getypeerde bloedplaatjessuspensie, werd gezien bij 87% van de patiënten, terwijl de 16-uurs transfusie opbrengst succesvol was bij 80% van de patiënten. Opvallend is dat bij een aantal van de refractaire patiënten bij wie geen HLA antistoffen konden worden aangetoond, toch enige opbrengst van HLA-getypeerde trombocyten werd waargenomen. Dit zal in prospectief onderzoek verder uitgezocht moeten worden.

Geconcludeerd moet worden dat bijna 90% van de patiënten die refractair zijn voor bloedplaatjes van willekeurige donoren, getransfundeerd kan worden met HLA-getypeerde bloedplaatjes. Bij refractaire patiënten met HLA-antistoffen die geen effect vertonen van HLA-getypeerde bloedplaatjes moet gezocht worden naar niet-immunologische factoren als oorzaak voor het afwezige transfusie effect. Indien deze niet aanwezig blijken te zijn moet onderzoek naar de aanwezigheid van bloedplaatjesspecifieke antistoffen overwogen worden.

Trombocytopenie veroorzaakt door aanwezigheid van autoantistoffen.
Immuun trombocytopenische purpura (ITP) is een ziekte waarbij het lichaam antistoffen maakt tegen de eigen bloedplaatjes (autoantistoffen) waardoor deze vroegtijdig worden afgebroken, met name in de milt. Het gevolg hiervan is een verkorting van de bloedplaatjeslevensduur hetgeen kan leiden tot een tekort aan circulerende bloedplaatjes en mogelijk een bloeding. Om dit te compenseren is het aantal megakaryocyten in het beenmerg meestal verhoogd waardoor de aanmaak van bloedplaatjes toeneemt.

Er is geen specifieke test voor het stellen van de diagnose. Evenmin zijn er klinische bevindingen die specifiek zijn voor de aandoening. ITP is daarom een diagnose bij uitsluiting.
Kinetisch onderzoek van bloedplaatjes is mogelijk door ze te labelen met radioactief indium. De bloedplaatjeslevensduur, turnover en de productie van bloedplaatjes blijken per patiënt soms sterk te variëren. Terwijl in het klassieke geval de levensduur zeer kort en de productie van bloedplaatjes sterk verhoogd is, blijkt er een subgroep van ITP patiënten te zijn met een verlaagde tot hoogstens normale productie en een slechts matige verkorting van de levensduur van de bloedplaatjes. Tevens blijken er 3 typen sekwestratiepatronen te bestaan, namelijk één type waarbij de bloedplaatjes in de milt te gronde gaan, een ander type waarbij de destructie vooral plaatsvindt in de lever en een derde type met destructie van bloedplaatjes diffuus in het mononucleaire-fagocyten systeem.
Hoe vaak de ziekte voorkomt is niet precies bekend, maar schattingen omtrent de incidentie lopen uiteen van 1.6 tot 6.6 gevallen per 100.000 per jaar (3).
Reeds enkele decennia zijn corticosteroiden de standaardbehandeling voor patiënten met ITP. Hoewel in veel gevallen een gunstig effect van corticosteroiden wordt waargenomen, blijkt dit vaak niet duurzaam en moet vervolgbehandeling ingesteld worden. Dit is meestal het verwijderen van de milt (splenectomie). Splenectomie resulteert in ongeveer 70% van de gevallen in een langdurig verdwijnen van de ziekte (remissie). Splenectomie is echter een invasieve ingreep en heeft een (klein) risico op infectieuze complicaties. De behandeling van patiënten die onvoldoende reageren op splenectomie is veelal problematisch.

Voor de vorming van antistoffen zijn B- en T-lymfocyten, die behoren tot de groep van witte bloedcellen (leukocyten), verantwoordelijk. Het werkingsmechanisme van corticosteroiden berust o.a. op onderdrukking van de aanmaak van de autoantistoffen die de trombocytopenie veroorzaken. Aangenomen dat zowel B- als T-lymfocyten betrokken zijn bij de productie van autoantistoffen, zou het de moeite waard kunnen zijn de antistof productie sterker te onderdrukken door prednison te combineren met een krachtig anti-T cel agens zoals bijvoorbeeld cyclosporine A.
Dit concept werd onderzocht in een prospectieve studie bij 20 patiënten met corticosteroid-refractaire ITP. De resultaten van deze studie zijn beschreven in **hoofdstuk 4.** Cyclosporine A werd gegeven aan tien patiënten die nog in het bezit waren van de milt (groep A), in de hoop dat bij een gunstig effect van de prednison-cyclosporine combinatie, splenectomie voorkomen zou kunnen worden. Bij de andere tien patiënten in de studie (groep B) was de milt reeds verwijderd, echter zonder voldoende resultaat. Ruim de helft (55%) van de patiënten reageerde gunstig op de combinatie van cyclosporine A en prednison. Echter, na gemiddeld 18 maanden ondergingen negen van de tien patiënten in groep A toch een miltextirpatie wegens onvoldoende of slechts tijdelijk effect van de cyclosporine.
Hoewel de resultaten zijn verkregen in een relatief kleine groep patiënten, lijkt behandeling met cyclosporine A splenectomie niet te kunnen voorkomen maar slechts tot uitstel te leiden. Of de recent geïntroduceerde immunotherapie in de vorm van anti-CD20 monoklonale antistoffen hierin verbetering zal brengen wordt momenteel onderzocht in een landelijke studie in HOVON verband. De voorlopige resultaten in de literatuur lijken veelbelovend.

Cyclosporine A is te overwegen bij moeilijk behandelbare patiënten met corticosteroid, anti-CD20 of splenectomie-refractaire ITP.

De rol van trombopoëtine bij autoimmuun trombocytopenie.
Het mechanisme van immuun trombocytopenische purpura (ITP) is nog niet volledig opgehelderd. Jarenlang werd aangenomen dat vroegtijdige destructie van met autoantistoffen beladen bloedplaatjes in de milt de enige oorzaak was van ITP. Echter, reeds in het begin van de tachtiger jaren toonden bloedplaatjeskinetische studies dat bij een aantal patiënten de bloedplaatjes productie normaal of verlaagd was in tegenstelling tot een verwachte verhoogde productie. Dit patroon past bij een gestoorde trombo- en megakaryopoëse.
De hematopoietische groeifactor trombopoëtine (TPO), vervult een belangrijke rol bij de productie van megakaryocyten en bloedplaatjes. Meting van de concentratie van TPO in het bloed, is nuttig gebleken om een aanmaakstoornis te kunnen onderscheiden van verhoogd verbruik van bloedplaatjes. In het eerste geval is de TPO concentratie in het bloed namelijk sterk verhoogd, terwijl de TPO concentratie in het laatste geval normaal of licht verhoogd is.
Om meer inzicht te krijgen in de regulatie van de megakaryo- en trombopoëse bij ITP, bestudeerden wij twee aspecten van de trombopoëse bij ITP, namelijk de serum TPO concentratie als maat voor de bloedplaatjes productie en de bloedplaatjes turnover, gemeten met behulp van radioactief indium-gelabelde autologe bloedplaatjes.

Hoofdstuk 5 vermeldt de resultaten van serum TPO metingen en bloedplaatjeskinetisch onderzoek bij 35, respectievelijk 15 patiënten met ITP. Bij alle patiënten lag de serum TPO concentratie binnen normale grenzen. Opvallend was dat de patiënten met een zeer laag bloedplaatjes aantal ($\leq 20 \times 10^9$/l) een significant hogere serum TPO spiegel hadden dan de patiënten bij wie het bloedplaatjesgetal boven de 20×10^9/l lag. Bloedplaatjeskinetische studies toonden een positieve correlatie (R=0.64) van serum TPO spiegels en de logaritme van de bloedplaatjes turnover. In combinatie met de uitkomst van de TPO metingen is dit suggestief voor een gestoorde regulatie van de megakaryopoëse bij ITP tengevolge van een (relatief) tekort aan TPO in het lichaam. De eerste resultaten van recent gestart onderzoek naar het effect van TPO behandeling bij patiënten met ITP zijn bemoedigend (4).

Trombocytopenie ten gevolge van bloedplaatjesconsumptie op basis van een onderliggende immuunstoornis.
Trombotische trombocytopenische purpura (TTP) is een bloedplaatjesconsumptie type trombocytopenie waarbij aggregaten van bloedplaatjes worden gevormd die de microcirculatie verstoppen. Trombotische trombocytopenische purpura (TTP) is een zeldzame, levensbedreigende, aandoening met een geschatte incidentie van 3.7/1.000.000 (5). TTP wordt gekarakteriseerd door een vijftal symptomen die meestal niet allemaal tegelijk aanwezig zijn. Het betreft de volgende symptomen en afwijkingen: een bijzondere vorm van bloedarmoede (microangiopathische hemolytische anemie), trombocytopenie, nierfunctiestoornissen, neurologische verschijnselen en koorts.
Trombocytopenie is het gevolg van bloedplaatjes klontering ten gevolge van de aanwezigheid van abnormale stollingsfactoren, de zogenaamde extra grote von Willebrand factor

multimeren, in de circulatie. Deze multimeren worden onder normale omstandigheden gekliefd door een specifiek eiwit, het von Willebrand factor klievend protease (ADAMTS13). Patiënten met de verkregen vorm van TTP vormen autoantistoffen tegen het ADAMTS13 eiwit waardoor de werking wordt geremd. Hierdoor kunnen deze extra grote von Willebrand factor multimeren niet gekliefd worden en blijven zij als zodanig in de circulatie.
De behandeling van TTP bestaat uit plasma wisseling met vers bevroren plasma, meestal in combinatie met immuunsuppressie in de vorm van corticosteroiden. Dit leidt tot remissie in 70-80% van de gevallen. De behandeling van patiënten met plasma-refractaire TTP en van patiënten met recidiverende ziekte is problematisch.
Op empirische gronden is men in het verleden in deze situaties soms overgegaan tot verwijdering van de milt (splenectomie). De ingreep werd veelal in de acute situatie uitgevoerd en ging vaak gepaard met complicaties, in een aantal gevallen zelfs met dodelijke afloop.

Wij onderzochten de waarde van splenectomie bij 33 patiënten met plasma-refractaire (9 patiënten) of recidiverende TTP (24 patiënten). De resultaten van dit onderzoek zijn beschreven in **hoofdstuk 6**. Splenectomie leidde tot een klinische remissie in op één na alle gevallen. De remissies hielden stand gedurende de vervolgduur van gemiddeld 69 maanden (uitersten 33-199 maanden). Negen patiënten kregen één of meer recidieven. In de periode na splenectomie traden 0.09 recidieven per patiënten-jaar op. De 10-jaars recidief-vrije overleving bedroeg 70% (95%-BI:50-83%). Bij patiënten met recidiverende TTP daalde het aantal recidieven van 0.74 per patiënten-jaar vòòr splenectomie, naar 0.10 recidieven per patiënten-jaar na de splenectomie.
Wij concludeerden dat splenectomie effectief en relatief veilig is wanneer de ingreep wordt uitgevoerd in een stabiele fase van de ziekte of in remissie. Splenectomie is te overwegen bij patiënten met plasma-refractaire en recidiverende TTP voor wie geen alternatief voorhanden is. Onderzoek naar de rol van de milt bij TTP is noodzakelijk om een betere selectie van patiënten voor splenectomie mogelijk te maken.

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CHAPTER 9

PUBLICATIONS

TOT SLOT

CURRICULUM VITAE

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TOT SLOT

Tot slot ga ik het huis verlaten waar ik bijna 30 jaar heb gewoond en waaraan ik de beste herinneringen zal bewaren. Het grootste deel van mijn tijd heb ik moeten besteden aan de patiëntenzorg, reden waarom mijn onderzoek voornamelijk klinische observaties betreft. Ik bedank hierbij de vele patiënten die bereid waren aan de diverse onderzoeken mee te doen.

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CURRICULUM VITAE

Maria Catharina Kappers-Klunne

geboren te De Bilt.

1960	diploma Gymnasium β, Thorbecke Lyceum Utrecht
1960 – 1969	studie geneeskunde, Rijksuniversiteit Utrecht
1963	stage inwendige geneeskunde (endocrinologie), Parijs, Frankrijk
1969	arts examen internship inwendige geneeskunde, Metropolitan Medical Center, St Barnabas Hospital Minneapolis, Minnesota, USA
1969 – 1975	specialisatie inwendige geneeskunde, Academisch Ziekenhuis Rotterdam-Dijkzigt (opleider Prof.dr. J. Gerbrandy) St Elisabeth Ziekenhuis, Haarlem (opleider Dr. L. van Beugen) St Elisabeth Ziekenhuis, Tilburg (opleider Dr. V.A. Terwindt)
1975	registratie als internist
1976	staflid afdeling hematologie, Academisch Ziekenhuis Groningen (hoofd Prof. Dr. H.O. Nieweg)
1977 – 1979	afdeling oncologie, Dr Daniel den Hoed Kliniek, Rotterdam
1979 – heden	staflid afdeling hematologie, Academisch Ziekenhuis Rotterdam-Dijkzigt, later Erasmus Medisch Centrum Rotterdam (hoofd Prof.dr. J. Abels, later Prof.dr. B. Löwenberg)