Immune thrombocytopenia: exploring antibodies, scintigraphy and immune modulation. Moving towards a new era for patients with ITP

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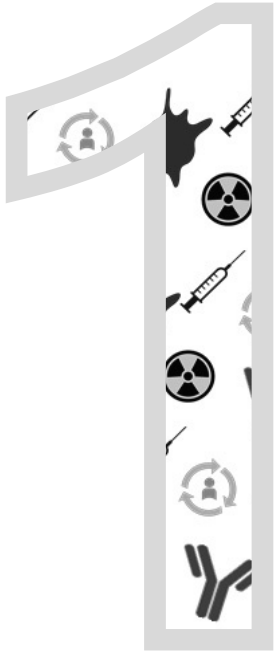

Chapter 1

General Introduction

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Immune thrombocytopenia

Immune thrombocytopenia (ITP) is an auto-immune disorder characterized by solitary thrombocytopenia under 100 x 10^9/L (reference range 150 – 400 x 10^9/L). (1) The large majority of ITP is idiopathic, whereas only in 20% of the patients the thrombocytopenia is linked to an underlying condition. (1, 2) The thrombocytopenia results from increased clearance of platelets combined with impaired production of platelets. Clinical signs of immune thrombocytopenia differ widely between patients. (3, 4) ITP is often diagnosed by deep thrombocytopenia and bleeding symptoms, including sometimes gastro-intestinal or intracranial hemorrhages. (4) However, most patients have little to no clinical bleeding symptoms and have mild to moderate thrombocytopenia. In general, bleeding symptoms are associated with thrombocytopenia with platelet count lower than 10 x 10^9/L. (5) There is an strong association between ITP and Quality of life. Patients with ITP are predicted to lose up to 20 years of quality-adjusted life years of their potential life expectancy. (4)ITP has an incidence rate of 1.6 to 3.9 per 100,000 patient years, is more present in females and increases with age. (5)

ITP can be divided into three categories, newly diagnosed, persistent, or chronic. (1)

- Newly diagnosed – Up to three months since diagnosis;
- Persistent – Three to 12 months since diagnosis;
- Chronic – More than 12 months since diagnosis.

Another division of ITP is based on the clinical presentation and categorizes patients into a mild, moderate and severe disease. Severe ITP refers to ITP with bleeding symptoms sufficient to require treatment; this typically occurs when platelet counts are below 30 x 10^9/L. (1) However, because the association between platelet count and bleeding severity is not straightforward, it might be more relevant to assess disease severity by the severity of the clinical symptoms and the requirement of new or additional therapies. (1)

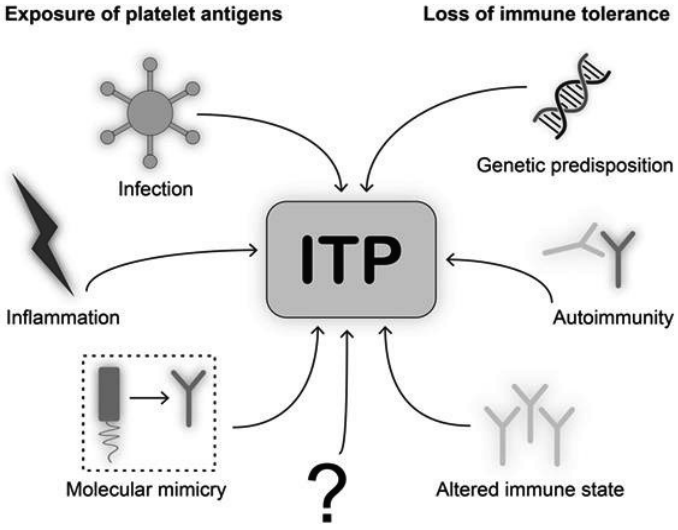
Pathophysiology and the role of anti-glycoprotein antibodies

Antibody-mediated destruction of thrombocytes is the central hypothesis of thrombocytopenia in ITP. The initiating mechanism behind platelet antigens and loss of immune tolerance is relatively poorly understood. T-cell imbalances may cause loss of self-tolerance and therefor start off B-cell mediated antibody-production is gaining recognition. (6, 7) Other hypotheses include CD4+ and CD8+ T cell mediated mechanisms, regulatory T- and B cells, antigenic cross-reactivity (molecular mimicry i.e. with the helicobacter pylori as pathogen), defects in the elimination of autoreactive B cell clones, and many more. (7, 8) Finally, CTL dependent platelet destruction might play a role. (9)

Figure 1 shows two important factors of ITP initiation. On the left exposure of platelet antigens is shown, like in the case of infection, inflammation and molecular mimicry. On the right processes in loss of self-tolerance are shown, like genetic predisposition, auto-immunity and altered immune state (such as after organ transplantation). However, this

figure contains also a question mark, illustrating there are ways we still don't understand in the ITP pathophysiology.

Figure 1 – Pathophysiology by Swinkels et al. 2018 Emerging Concepts in Immune Thrombocytopenia. Frontiers in immunology, 9, 880.



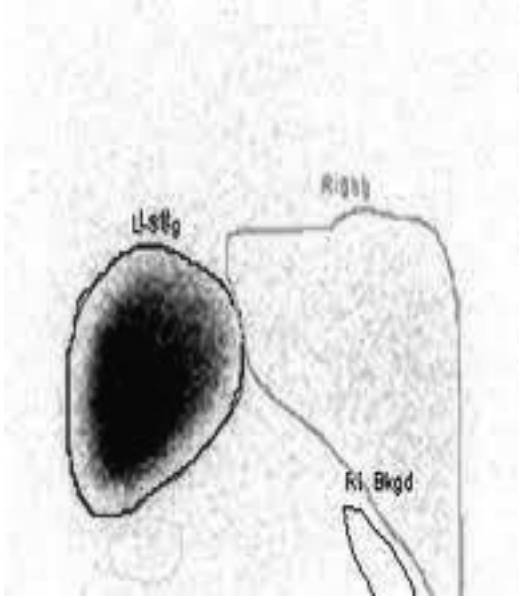
In the majority of ITP patients antibodies against membrane bound glycoprotein (GP) are found. These IgG auto-antibodies, produced by B cells, are usually directed against glycoproteins on the platelet membrane, such as glycoprotein IIb/IIIa, Ib/IX and V. The distribution of antibody types in ITP patients is as follows: anti-GP IIb/IIIa is found in 43%-71%, anti-GP Ib/IX is found in 64-80% and GPV it is 65-83%. (10, 11) Autoantibodies accelerate platelet clearance through removal by splenic macrophages and dendritic cells, complement deposition and platelet apoptosis. (12, 13) Phagocytes bearing Fcγ-receptors (FcγRs) in the spleen recognize platelets opsonized with antibodies resulting in antibody-mediated platelet phagocytosis. (7) The clearance of platelets takes place in both the spleen and liver. (11, 14) In this regard, it is hypothesized that this hepatic clearance may be induced by anti-GPIb/IX leading to accelerated desialylation of platelets. Ashwell Morell receptors may recognize platelets, which may lead to an increased clearance rate in the liver compared to healthy individuals. (7, 15, 16) Besides platelet destruction, an inhibited platelet production due to anti-megakaryocyte antibodies may play a role in ITP. (8, 11)

The diagnostic value of antibody testing is reviewed in several systematic reviews. Porcelijn et al. stated that antibody detection may contribute to diagnosing ITP. These relatively new assays show good results in terms of sensitivity and especially specificity. (17) GP-specific testing using direct and indirect MAIPA methods (Monoclonal Antibody-specific Immobilization of Platelet Antigen) had a sensitivity of 81% and specificity of 98% for identification of antibodies in ITP compared to analyses using PIFT (platelet immunofluorescence test); and can also be used to follow-up antibody levels in time. (18)

Pathophysiology and prognosis: the role of autologous platelet scintigraphy

Despite some promising new diagnostic tools, such as antibody testing, ITP remains a diagnoses per exclusionem in patients with isolated low platelet (i.e. without anemia or leukopenia). (1) At the present time, no robust clinical, laboratory or radiological parameters are available to confirm the diagnosis with high certainty (1, 19). Indeed it is important to exclude other explanations for thrombocytopenia and to identify conditions that contribute to or cause ITP. (1, 19, 20) In this regard, immune suppression should be applied with extreme caution in case of a concurrent infection. Other causes of thrombocytopenia, however, will most likely become apparent via thorough history taking, clinical examination and laboratory results. (18, 19) Radiologic or standard bone marrow examination are not needed for the diagnosis of ITP at this moment. However, when the patient has chronic or therapy refractory ITP additional diagnostics are needed. E.g. when splenectomy is considered as therapeutic option, imaging techniques using nuclear agents, such as the 111-indium, are able to visualize and quantify if platelet sequestration is predominantly splenic, hepatic or mixed. (21, 22) This clearly will aid the decision making process before undergoing a splenectomy. Most important in this regard is that some studies suggest splenic sequestration to be associated with a positive outcome after splenectomy. (13, 14)

Figure 2 – Indium-111 labelled platelet scintigraphy scan. S:L ratio 89% splenic to 11% hepatic sequestration. Results from Haga Teaching Hospital



The 111-Indium labeled sequestration study can be used to categorize the site of platelet sequestration in ITP patients. (21, 23) It is performed in accordance with the recommendations of The International Committee for Standardization in Hematology Panel on Diagnostic Application of Radionuclides. (23) The results of the scan uses a spleen to liver (S:L) ratio to categorize the patients into three categories: a predominantly splenic, hepatic or mixed pattern. Categorization of platelet sequestration patterns was adopted from Najean et al (23) where scan outcome is a ratio between liver and spleen in percentages at 30 minutes, 24 hours and 48 hours after reinjection of labeled

platelets. The use of the Indium-111 scan may lead to new insights in pathophysiological pathways, and as a prognostic tool for a splenectomy. (13, 14)

Treatment and the role of TPO-receptor agonists

ITP treatment consists of both immune suppressant drugs, thrombopoietin agonists and splenectomy. First line treatment is initiated when platelet counts fall below 30 $x10^9$/L, or when patients have clinically relevant bleeding. Emergency treatment usually starts with high dose corticosteroid treatment and intravenous immunoglobulins (IVIg). This first line treatment however, is often not curative and come with substantial side-effects and risks. (20) In the Netherlands, second line therapies are either anti-CD20 therapy (Rituximab), or thrombopoietin receptor agonists (TPO-receptor agonists), whereas performing a splenectomy moved up to a third line treatment. (27) When these options fail, combination therapy can be used, for example: the combination of Rituximab with dexamethasone.

TPO-receptor agonists

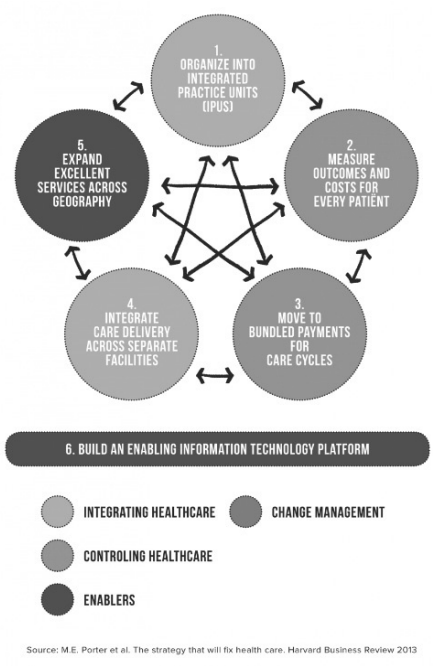
TPO-RAs have gained an important role in the management of ITP. At the moment romiplostim, eltrombopag and avatrombopag are TPO-RA's available in Europe. (24) These drugs bind to the TPO receptor (c-MpL), which leads to stimulation of megakaryocyte differentiation and proliferation. (24, 25) Romiplostim is a recombinant, Fc-peptide fusion protein (peptibody) given subcutaneously as a weekly dose, while eltrombopag and avatrombopag are daily oral drugs that bind to the transmembrane region of c-MpL. (25, 26) The effectivity of TPO-RAs for response in platelet count is 69-75% and the side-effects are well-tolerated and reversible after discontinuation of the drugs. (25-27) Most described side effects consist of headache, dizziness, and possibly an increased risk of thrombosis. (25, 26) Interestingly, TPO-RA seem to have an immune modulatory effect as well. This immune modulation might involve changing regulatory T cell numbers and function. (28-30) This makes TPO-RA therapy even more interesting namely as a potential cure for ITP. As with all therapeutic approaches, responder vs non-responder comparisons should identify mechanistic biomarkers to monitor and hence also predict the effectivity the best therapy for a specific ITP patient.

Implementation of research, patient reported outcomes, guidelines and decision-making

In a chronic illness, such as ITP, it is important to translate new scientific work to clinical practice in both guidelines and decision making tools. Furthermore, patient reported outcomes can guide new scientific work to improve the quality of life of ITP patients.
The last chapter of this thesis discusses the implementation of the scientific work into clinical practice. Value-based health care (VBHC) is a framework for healthcare systems and aims to get the highest value for patients at lowest cost. (31) Value in this sense is defined

by patient reported outcome divided by cost. This concept is introduced by Porter et al in 2006. To enhance higher value the system needs not only to lower cost, but also to aim for higher outcomes. Many healthcare workers over the globe are working to change the systems toward patient reported outcomes and experience instead of medical achievements only, VBHC initiatives could aid in achieving these goals.

Figure 3 – Value Based Health Care concept



Measuring outcomes via PROMs

Currently, the main outcome that is discussed in the outpatient clinic is the platelet count. Patient reported symptoms, which can be measured using patient reported outcome measures (PROMs) are still in development. In a collaboration with hematologists, pharmacist and patient representatives via the Dutch patient organization we aimed to set up an ITP PROMs set. Our aim is to implement this in the Haga teaching hospital from 2022 and make the set available to other hospitals in the Netherlands.

Decision-making tools

Most ITP patients find it hard to make an informed decision. Decision making tools could aid patients and caregivers to discuss the potential treatment options and improve informed decision making. We aimed to develop an internet-based decision tool for patients with chronic ITP in close cooperation with the Dutch patient organization.

Guideline in the Netherlands

The national guideline for the diagnosis and treatment of ITP in the Netherlands was outdated and is updated in 2020 including the newest insights on TPO-RA, described the added value of the scintigraphy and platelet antibodies. Moreover, it gave a start to with incorporating patient views through the implementation of PROMs for Dutch ITP patients.

Outline and aims of this thesis

This thesis aims to investigate the following themes and questions on ITP:

1. Pathophysiology of platelet destruction: What is the role of anti-glycoprotein antibodies in relation to the site of platelet sequestration and TPO level? Which tools can aid in treatment choice and response? What is the role of platelet scintigraphy?
2. Treatment options and immune modulation: Can TPO receptor agonists treatment lead to immune modulation and result in treatment free responses? Can we use TPO receptor agonists for peri-operative bridging?
3. Implementation and patient reported outcomes: Can we develop an ITP tailored patient reported outcome measure and decision making tool for Dutch ITP patients?

Pathophysiology | **Chapter 2 and 3** investigate the role of anti-glycoprotein (anti-GP) antibodies Ib/IX, IIb/IIIa,V and platelet sequestration site determined by autologous platelet scintigraphy. Chapter 3 further investigates the potential role of anti GPIb antibodies on TPO production and platelet clearance in the liver in a clinical population of chronic ITP patients. **Chapter 4** describes a short report of an interesting case of adalimumab (TNFα-inhibitor) induced ITP in which anti-glycoprotein antibodies were present, while other causes of thrombocytopenia were ruled out. This study could have implications for platelet monitoring during the use of TNFα inhibitors. **Chapter 5** is a systematic review and meta-analysis of the existing literature on autologous platelet scintigraphy and its association with clinical outcomes of splenectomy in ITP patients. Since platelet sequestration can occur in both the spleen and liver, the sequestration pattern might aid in the clinical decision-making of performing a splenectomy and possibly predicting the success rate of such an invasive procedure, ultimately reducing iatrogenic harm to patients. **Chapter 6** investigated the robustness other Indium-111 labeled platelet scintigraphy. These scans are not performed on a large scale and clinical data is scarce. This study used intraclass correlation and Cohen's kappa to assess robustness and interobserver reliability of the sequestration pattern results, thus providing evidence of the reliability and safety of these scintigraphy studies in clinical practice.

Treatment | **Chapter 7** systematically reviews and performs a meta-analysis on the risk of thrombosis of patients treated with TPO-RA compared to patients treated without TPO-RA. The primary outcome was the incidence of thromboembolic events. This study can aid in better understanding the safety and guide monitoring of TPO-RA in clinical practice. **Chapter 8** describes a protocol for an intervention trial which examines the use of TPO-RA in a clinical population of chronic ITP patients. The primary aim is to

investigate whether TPO-RA can be safely discontinued after a year of treatment while keeping an platelet count above 50 (treatment free remission, TFR).
A TFR may be reached by possible immune modulatory effects of the TPO-RA treatment. Furthermore, this study aims to investigate which patient characteristics are associated with successful stopping of TPO-RA. Results from this study could aid in personalizing treatment and preventing life-long TPO-RA treatment with its associated health costs.
Chapter 9 describes the Bridging ITP Trial, which is an open-label randomized trial comparing TPO-RA Eltrombopag to intravenous immune globulin (IVIg) in ITP patients who require an increase in platelet count before surgery. This study is a multicenter trial in both Canada and the Netherlands. This trial can provide evidence on the safety and efficacy of eltrombopag in the peri-operative setting for patients with ITP.

Implementation | **Chapter 10** describes several initiatives to improve healthcare for Dutch ITP patients. First, the Dutch clinical guideline for ITP was updated and a clinical article highlighting the most important changes has been published in the Dutch Hematology Journal. Furthermore, a PROM was developed and tested in close cooperation with the Dutch ITP patient association to aid patients and clinicians to discuss outcomes most relevant from a patients perspective. Lastly, a clinical decision aid for ITP patients was developed on the platform 'Keuzehulp.info'.

Discussion, interpretation and future | **Chapter 11** discusses and interprets all research described in this thesis. This will be related to clinical implications and suggestions for future research will be provided, including recommendations to improve healthcare and value based healthcare initiatives.

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