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EPIDEMIOLOGICAL PERSPECTIVE OF CHRONIC IMMUNE THROMBOCYTOPENIA

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THROMBOCYTOPENIA
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family for always believing in me.

"Everything will be okay in the end, if it's not okay it's not the end".

Unknown

ABSTRACT

This thesis deals with different aspects of immune thrombocytopenia disease (ITP), what happens before diagnosis, characteristics of patients at treatment start and outcomes potentially related to the disease and treatment, such as thrombosis and cancer.

In study one we investigate the risk of infections prior to the diagnosis of ITP. There are some infections causing secondary ITP and there is an association with infection and risk of autoimmune disease. With information from national health registries we were able to compare the amount of diagnosis of infection and anti-infective drugs within five years before diagnosis of ITP in 1087 patients with primary chronic ITP (cITP) to the general population. Our hypothesis turned out to be right and the patients with ITP were more likely to have had an infection diagnosis, Standardized Incidence Ratio (SIR) 8.74(7.47-10-18) and anti-infective drugs SIR 1.37(1.25-1.50) compared with persons of the same age and sex in the general population. In addition, we estimated the incidence of ITP in Sweden to 2.3 per 100 000.

In study two we investigated characteristics at treatment start in patients with (cITP). We wanted to know how the patients' characteristics influence start and type of treatment. We found that patients start treatment at low platelet counts, median 12(IQR 5-27). This finding supports the recommendation to treat in order to avoid symptoms and not aim for a normal platelet count, in order to avoid unnecessary potential harm from the treatment. Moreover, we confirm that the most common first treatment is corticosteroids followed by diverse treatment used as second line treatment and refractory treatment. During the last four years of the study splenectomy was less common and the time to splenectomy delayed. Comorbidity influenced treatment start and type of treatment. Patients with diabetes were less likely to receive corticosteroids.

In the third study underlying risk factors for arterial thrombosis and venous thromboembolism were evaluated. In collaboration with a research group at the university of Toulouse a cohort study was performed using the same variables and analysis in both countries. The incidence rate of arterial thrombosis in France was 15(13.4-16.7) and in Sweden 14.7(12.4-17.5) and for venous thromboembolism in France 6.9(5.9-8.1) and 6.5(5.1-8.1) in Sweden per 1000 person-years, 95% CI. The impact of baseline risk factors was similar as well.

In study four we studied the risk of cancer in patients with ITP. Molecular evidence suggests that the dysfunctional immune system related to autoimmune disease may increase the risk of certain cancers. A risk of haematological malignancies has been reported in studies of patients with ITP but the risk of solid tumours warrants further attention and those performed have reported contradictory results. We compared the rate of cancer in patients with ITP to the general population in a matched cohort study. We found a slightly increased risk of overall cancer HR 1.37(1.27-1.48), more pronounced in men, an increased risk of liver cancer 3.83(2.46-5.97) and an increased risk of skin cancer after 10 years of follow up, 1.52(1.05-2.19). We confirmed the increased risk of haematological malignancies. The risk of hematologic malignancies was increased in all time intervals and follow a trend with the highest risk, 9-fold, following the year of diagnosis of ITP to down to twofold higher 10-20 years after diagnosis We conclude that treating clinicians should have a high index of suspicion of cancer when treating these patients.

SAMMANFATTNING

Avhandlingen behandlar olika aspekter av sjukdomen immun trombocytopeni (ITP): händelser före diagnos, patientkaraktäristika vid start av behandling och sjukdomar som kan vara associerade med ITP-sjukdom och behandling såsom trombos och cancer.

I den första studien undersökte vi förekomst av infektioner innan ITP-diagnosen. Några infektioner kan ge sekundär ITP och det finns en koppling mellan infektion och risken för autoimmun sjukdom. Med information från nationella hälsoregister kunde vi studera förekomst av infektionsdiagnoser och infektionsläkemedel inom fem år innan ITP-diagnosen. Vi jämförde 1087 patienter med primär kronisk ITP med populationen. Vår hypotes stämde, patienter med ITP hade fler infektioner Standardized Incidence Ratio (SIR) 8.74(7.47–10-18) och infektionsläkemedel SIR 1.37(1.25–1.50) jämfört med individer i populationen av samma ålder och kön. Vi bestämde också incidensen av kronisk ITP till 2.3 per 100 000.

I studie två undersökte vi patientkaraktäristika vid start av behandling för kronisk ITP. Vi ville veta hur patientkaraktäristika påverkar start och typ av behandling. Vi fann att patienterna har låga värden på trombocyterna vid start av behandling, medianvärde 12(IQR 5-27). Detta fynd är i linje med rekommendationerna att behandla för att undvika symptom och inte sträva efter ett normalt trombocytvärde. Detta för att undvika onödig biverkan av behandlingen. Vi bekräftar att den vanligaste behandlingen att starta med är

kortikosteroider följt av olika val vid sekundär och refraktärbehandling. De senaste åren är splenektomi mindre vanligt och utförs senare i behandlingsförloppet. Komorbiditeter såsom diabetes påverkar behandlingsvalet och färre patienter med diabetes får kortikosteroider.

I studie tre undersöks riskfaktorer för arteriell trombos och venös tromboembolism. I samarbete med en fransk forskargrupp vid Toulouse universitet utfördes en kohortstudie med samma variabler och analysmetoder. Incidensen för arteriell trombos i Frankrike var 15(13.4–16.7) och i Sverige 14.7(12.4–17.5) och för venös tromboembolism i Frankrike 6.9 (5.9–8.1) och 6.5(5.1–8.1) i Sverige per 1000 personår med 95 % konfidensintervall. Således var riskerna lika stora i båda länderna. Påverkan av riskfaktorer såsom komorbiditeter var också liknande.

I studie fyra studerade vi ITP-patienternas risk för cancer. Det finns molekylära mekanismer som antyder att den dysfunktionalitet som förekommer i immunsystemet hos patienter med autoimmuna sjukdomar kan öka risken för vissa typer av cancer. Det finns studier som visat att patienter med ITP har en ökad risk för hematologisk cancer, studier på solida tumörer är få och de som finns har visat motsägande resultat. Vi jämförde risken för cancer i patienter med ITP mot en matchad kohort i populationen. Vi fann en viss överrisk för cancer totalt sett Hazard Ratio 1.37(1.27–1.48), mer uttalad i män. Det fanns en ökad risk för levercancer 3.83(2.46–5.97) och en ökad risk för hudcancer efter 10 års uppföljningstid 1.52(1.05–2.19). Vi bekräftade den ökade risken för hematologiska maligniteter. Den risken var ökad i alla tidsintervall, så hög som niofaldig under första året efter diagnos, och ner till tvåfaldig efter 10-20 år. Vår slutsats är att behandlande personal ska vara uppmärksamma på risken för cancer hos patienter med ITP.

LIST OF SCIENTIFIC PAPERS

I.   **Charlotta Ekstrand,** Marie Linder, Honar Cherif, Helle Kieler and Shahram Bahmanyar.
     Increased susceptibility to infections before the diagnosis of immune thrombocytopenia.
     Journal of thrombosis and haemostasis 2016; Volume 14, Issue 4: 807–14.

II. **Charlotta Ekstrand,** Marie Linder, Honar Cherif, Helle Kieler and Shahram Bahmanyar.
     Patient characteristics when starting treatment and patterns of treatment in adults with chronic immune thrombocytopenia.
     Accepted for publication in Blood, Coagulation and Fibrinolysis 27 July 2019.

III. **Charlotta Ekstrand,** Marie Linder Bérangère Baricault, Margaux Lafaurie , Laurent Sailler , Maryse Lapeyre-Mestre Helle Kieler, Guillaume Moulis and Shahram Bahmanyar .
     Impact of risk factors on the occurrence of arterial thrombosis and venous thromboembolism in adults with primary immune thrombocytopenia-Results from two nationwide cohorts.
     Thrombosis Research; Volume 178 Issue: June 2019; Pages 124-131.

IV. **Charlotta Ekstrand,** Shahram Bahmanyar, Honar Cherif, Helle Kieler and Marie Linder.
     Cancer risk in patients with primary ImmuneThrombocytopenia. Manuscript.

<u>List of scientific papers not included in this thesis</u>:

- Löfling Lukas, Linder Marie, **Ekstrand Charlotta**, Cherif Honar, Kieler Helle, Bahmanyar Shahram.
  Pharmacological agents preceding a diagnosis of immune thrombocytopenia in adult patients developing the chronic form: A Swedish national register study. Thrombosis Research Volume 160 Issue: December 2017 Pages 27-31.

- Adelborg K, Kristensen NR, Nørgaard M, Bahmanyar S, Ghanima W, Kilpatrick K, Frederiksen H, **Ekstrand C**, Sørensen HT, Fynbo Christiansen C.
  Cardiovascular and bleeding outcomes in a population-based cohort of patients with chronic immune thrombocytopenia.Journal of Thrombosis and Haemostasis 2019 Jun;17(6):912-924.

CONTENTS

List of abbreviations

ATC	Anatomic Therapeutic Chemical classification system
cITP	chronic Immune Thrombocytopenia
ICD	International Classification of Disease
IR	Incidence Rate
IRR	Incidence Rate Ratio
ITP	Immune Thrombocytopenia
IVIg	Intravenous Immunoglobulin
NPR	National Patient Register
PDR	Prescribed Drug Register
SIR	Standardized Incidence Ratio
SNDS	Système National des Données de Santé
TPK	Platelet count
TPO-RA	Thrombopoietin Receptor Agonist
TPR	Total Population Register

Introduction

Having a chronic disease means having to deal with the consequences of the disease, sometimes for the rest of one's life. It means to suffer from and to be aware of symptoms and potential risks linked to the disease, to depend on the effect and safety of drugs for a long time, and still maintain an everyday life. The aim of this thesis was to study some aspects of the disease immune thrombocytopenia, a disease where the majority of the patients develop a chronic disease. Every individual is unique and every patient may have certain characteristics that influence the course of the disease and the treatment. Watching from a helicopter perspective can make us realize patterns and understand our observations better than if it only one observation in one patient is analysed. Epidemiology can be that helicopter perspective and bring new perspectives to the clinic. Even though I have only read information about these patients in medical records and retrieved information from health registers it feels as if I almost know them a little after 10 years "together". I hope my work will be a piece in the puzzle of improving their care.

The overall aims of the thesis:

Study I.

☐To estimate the incidence of chronic ITP in adults in Sweden, diagnosed between 2007 and 2011.

☐To estimate the ratio of specific infections in patients with ITP compared with the general population, other than infections linked to secondary ITP, during a five years' period before the diagnosis of chronic ITP.

☐To investigate exposure specific drugs for infection in the year before ITP diagnosis.

Study II.

☐To assess predictors of ITP treatment start, in relation to platelet counts and characteristics of patients regarding age, sex, co-medication and comorbidity.

☐To describe treatment patterns in patients with cITP.

Study III

☐To determine the incidence rate of arterial thrombosis and venous thromboembolism in patients with ITP in France and Sweden.

☐To evaluate the impact of baseline risk factors on the risk of arterial thrombosis and venous thromboembolism from national health registries in France and Sweden.

Study IV.

☐To analyse the risk of hematologic cancer and solid cancer in patients with ITP compared with matched comparators in the general population.

1 BACKGROUND, CHAPTER 1

1.1 History of immune thrombocytopenia

Purpura is the Latin name for purple and is used in medicine to denote skin haemorrhages. Disease related purpura was discovered centuries back. One of the important milestones was achieved in 1025 when the Persian physician and philosopher Avicenna described chronic purpura and several centuries later the clinical definition was proposed and the course of ITP described by the German physician Paul Gottlieb Werlhof (1699–1767). The disease was named after him and at that point called "Werlhof disease". In 1951 doctor Dr. William J Harrington injected himself with plasma from a patient with immune thrombocytopenia (ITP) and developed thrombocytopenia with symptoms, and a thrombocytopenic factor in the blood was discovered. Since then ITP has been recognized as a thrombocytopenic disease (1, 2). The term platelet (Figure 1) was introduced in 1882 when the Italian pathologist Bizzozero demonstrated that platelets are an independent cell line with a role in thrombosis and haemostasis (3). The understanding of platelet function and the ITP-disease mechanisms grow more and more over the years, different research groups investigated the disease mechanisms and in the seventies and eighties the binding of Immunoglobulin G (IgG) to platelets and megakaryocytes was explained. Several studies have reported T-lymphocyte cell abnormalities since the early nineties (4).

Disease definition

Until 2009 the definition of ITP disease was thrombocytopenia with platelet counts less than 150×10^9/L. The disease was denoted acute ITP if the diseased lasted up to six months and chronic if the thrombocytopenia persisted more than six months. In 2009 both the name and the definition changed. An international working group decided to change the name from Idiopathic thrombocytopenic purpura to Immune thrombocytopenia (ITP) because it is established that the disease is autoimmune and not idiopathic and not all patients experience purpura. The acronym ITP was kept since it was so well established (5). The definition now requests platelets less than 100×10^9/L since some people can have low platelets in their natural state. Starting from 2009 the stages are defined in the following manner: newly diagnosed, persistent and chronic (Table 1). The new category persistent was added because some patients do not develop chronic ITP and it is not necessary to start heavy treatment and splenectomy in those patients who do not develop the chronic form of the disease (5).
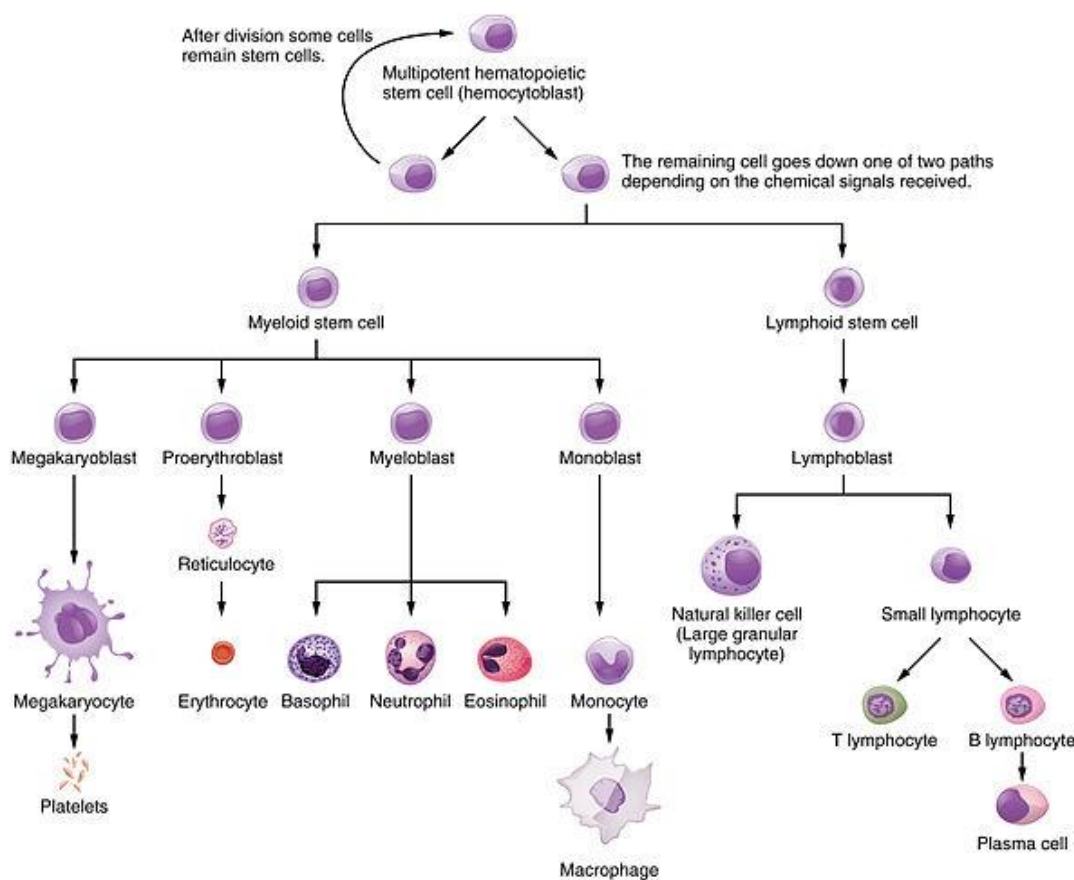
Figure 1. Haematopoiesis, platelet development.

1.2   Characteristics of Primary ITP disease

Primary immune thrombocytopenia can be a lifelong disease although some patients do experience disease remission in such a degree that they no longer need to be followed in a hospital setting. Chronic ITP is often a disease of remission and exacerbation, the severity of disease and need for treatment can vary for the same patient throughout the years. Symptoms can be both dry bleeding and wet bleeding including excessive bleeding during surgery or injury and intracranial bleeding (6) (7, 8). Patients with ITP need extra surveillance at dentist interventions, during surgery, pregnancy and delivery. Platelet levels can be chronically low and some patients need maintenance therapy in order to keep a safe level. It is now consensus that a safe platelet level is a level without risk of symptoms of bleeding rather than a normal platelet count level. Normal platelet count levels seem to differ between different geographical regions and that is one of the reasons why the international working group changed the definition of thrombocytopenia in ITP from 150 to

100 *10^9/L. Studies performed in European countries have reported that a normal platelet count for women is around 169–358 *10^9/ L and for men 143–332*10^9/L whereas a study in Africa showed a lower platelet counts 125-342 x10^9/ L for females and in male 115-290 x10^9/L (15). Platelet counts over 30 *10^9/L is usually considered safe. Furthermore other factors influence the risk of bleeding and accordingly the need for treatment ,such as comorbidity, age and lifestyle (5, 6).

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Table 1. Primary ITP, definition

•An autoimmune disease with increased platelet destruction and relatively decreased platelet production.

•Platelets threshold, less than 100 x10^9/L.

•Possible causes of the thrombocytopenia has been examined and no cause found.

•Normal bone marrow or increased amount of megakaryocytes.

•Symptoms include bleeding which can be dry (e.g. petechiae, hematoma) or wet bleeding (e.g. epistaxis, gastrointestinal and intracranial bleeding).

ITP phases

•Newly diagnosed less than 3 months.

•Persistent 3 to 12 months.

•Chronic over 12 months (5, 6).

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Primary versus secondary ITP

The disease can be either primary or secondary. Secondary ITP is when the thrombocytopenia is secondary to an underlying cause, such as an infection or exposure to a drug (Table 2), it is the underlying condition that needs to be treated or the drug that needs to be discontinued in order to treat the thrombocytopenia (9, 10). In other words, it has clinical relevance to know if the thrombocytopenia is primary or secondary. The aetiology behind primary thrombocytopenia is unknown and the treatment is focused on keeping the platelets at a safe level to avoid bleeding. One way to obtain a safe level is by modifying or suppressing the underlying immunological reaction in order to inhibit the production of autoantibodies or hinder them from attacking the platelets, another is stimulation of the production of platelets in the bone marrow (6).

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Table 2. Causes of secondary ITP

•Drugs e.g. heparin and quinidine.

•Excessive alcohol consumption.

•Infections: HIV and viral hepatitis (B or C).

•Haematological malignancies (mainly CLL and lymphomas).

•Autoimmune lymphoproliferative syndrome type I (ALPS).

•Antiphospholipid syndrome.

•Autoimmune diseases: Rheumatoid Arthritis, Systematic Lupus Erythematosus, Evans syndrome (i.e. ITP and Autoimmune haemolytic anaemia)

(8),(10)

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1.3 ITP Epidemiology

A French study reported the incidence according to the new definition to 1.61 per 100 000/year (11). The estimated incidence (according to the old definition) is in the range 1.6–3.9 per 100 000/year reported in a systematic review. ITP is more common in the middle aged and marginally more dominant in females (ratio 1.2-1.7). Sex differences are not seen in children and in the elderly. Most studies about incidence and prevalence have been performed in western countries and are not differentiating between different stages of ITP. In the systematic review, previously cited, all studies were European, except one, which that was from Kuwait (12). A study from Japan reported an incidence for primary ITP, according to the old definition, to be 2.16 per 100,000/year. The difference between males and females, was larger than reported elsewhere 1.7 and 2.6, respectively.

1.4 Aetiology and pathogenesis of primary ITP

All the blood cells come from a pluripotent hematopoietic stem cell. In the bone marrow the megakaryocytes are precursor cells that differentiate into platelets. A lymphoid progenitor cell differentiates in the blood to T-cells and B-lymphocytes. The lymphocyte cell line is supposed to have a specific attack towards foreign antigen and not an unspecific attack towards the body's own platelet cells, which is the case in the autoimmune reaction of ITP (13).The aetiology behind the development of autoimmunity in primary ITP is to a large extent unknown. Certain disease mechanisms are known, such as autoimmune increased destruction of platelets and reduced production of platelets. ITP is now considered a T-helper lymphocyte cell disease characterized by the activation and accumulation of T-helper cells (14) (15). The Tregs, T-cells that carry the co-receptor protein CD4+, are defect. Considering that these cells differentiate into T-helper cells that

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activate macrophages and B-cell responses to antigen and other important regulatory lymphocytes, this dysfunctionality plays an important role in the loss of self-tolerance in patients with ITP (16, 17). The Tregs are decreased and less functioning both in the spleen and the circulation (18). Experimental studies demonstrated that Tregs were decreased in the spleen in patients with ITP compared with controls (19). Several studies have shown the decreased or defective function of Tregs in patients with ITP. These cells suppress the activation and proliferation of many cell types including T-cells, B-cells, dendritic cells and natural killer cells (NK). The Tregs are important because they control haemostasis and immunopathology. Defective Tregs in patients with ITP can be one reason to the loss of immunologic self-tolerance and pathogenesis in these patients. In other words, there is dysfunctionality in important regulatory cells needed for a functional immune response (20).

The destruction of the platelets is caused by autoantibodies targeting the glycoproteins GPIIb/IIIa, GPIb/Ix and GPIa/IIa causing opsonisation of the platelets and phagocytosis of macrophages in the spleen. The autoimmune attack from autoantibodies and CD8+ T cells directed towards megakaryocytes as well as the TPO hinder a compensation of platelet production resulting in thrombocytopenia (18).

1.5 Genes

ITP is not regarded as an inherited disease. There are a couple of studies that have shown similarities in gene expression between unrelated ITP-patients, Single Nucleotide Polymorphism, which can give rise to different expression of the gene, arises when there is a difference in a single nucleotide in the DNA. Common polymorphisms have been reported in the Fc-receptor in unrelated ITP-individuals. The Fc-receptor is located on chromosome 1q23-24. The receptor binds to the Fc region on Immunoglobulin G. The expression of this gene variant leads to an altered balance of inhibitory and activating regulator FcγR on immune cells. Fc gamma is a regulator that activates IgG and the result of having this allele (a one base different gene variation) is unbalanced immunity and auto-inflammation. In a study comparing 116 patients with ITP to 100 healthy controls the FCGR2C-ORF allele was overrepresented among the patients with ITP (18.9% in ITP versus 8.8% in healthy controls). The control group had a SNP (Single Nucleotide Polymorphism) in the FCGR2C allele as a stop codon so IgG was not activated. The conclusion was that the FCGR2C-ORF allele predisposes to ITP (21).

A Swedish group performed a gene expression study on bone-marrow derived T-cells from patients with primary cITP and compared them to non-ITP individuals. The patients with

cITP had altered expression in T-cell pathways involved in immune functions necessary for a functional immune response. The findings indicate a non-functional immune response in bone marrow derived cells from patients with ITP compared with non-ITP individuals (21, 22).

Familial aggregation of ITP has been shown. In a small study, which included paediatric patients with a diagnosis of primary ITP, 2.3% of 445l patients had a positive family history (23).

1.6 Autoimmunity and infection

How the immune system loses its specificity and starts to turn on its own cells is a complicated disturbance in the immune system. In the normal state, the body's innate and adaptive system handles virus and bacteria and when the infection has run its course the immune cells decrease and there is no attack on the body's own cells (13). The pathogenesis between infection and autoimmune diseases is not completely clarified. Several hypotheses are being evaluated. One of them is the "molecular mimicry", when the antigen mimics a self-peptide, a cross-reaction occurs, and the autoimmune responses is directed towards the self-molecule. Another one is "protein changes, cryptic antigens" which can happen during infection when antigens that should be recognized as self becomes non-self and starts an autoimmune response. "Super-antigens" can trigger a number of lymphocytes to react without specific binding. "Bystander activation" is yet another mechanism in which where there is epitope-spreading of the antigens resulting in a large activation of lymphocytes (24).
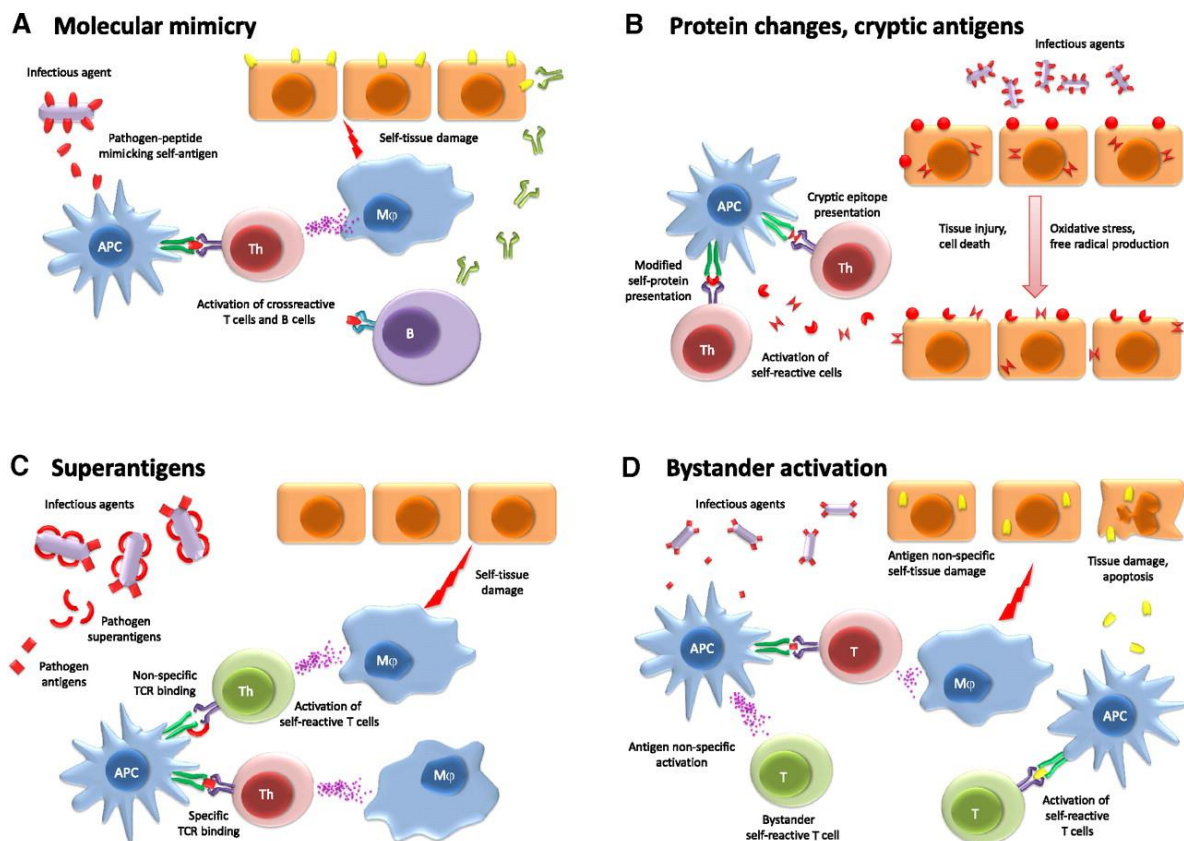
Fig 1. Autoimmune mechanisms induced by pathogens. Used with approval of Sfriso P, Ghirardello A, Botsios C, Tonon M, Zen M, Bassi N, et al. Infections and autoimmunity: the multifaceted relationship. Journal of leukocyte biology. 2010;87(3):385-95.

Previously mentioned, we know from other studies that ITP-patients show alterations in genes involved in immune pathways (FCGR2C gene, IL1RN VNTR and IL2-330), polymorphic genes compared to those of healthy individuals (21, 25). These alterations disturb the natural pathway of the immune response and the altered immune response cannot handle infections like they should. Dysregulation of T cell activity and cytokine abnormalities is a mechanism in active ITP and furthermore contribute to other autoimmune diseases. Altered levels of T-helper cell 17-related cytokines are linked to the induction of autoimmune diseases like rheumatoid arthritis (RA) as well as in the pathogenesis of ITP (26). There are findings of similar alterations in several different autoimmune diseases e.g. ITP, RA and systemic lupus erythematosus (SLE) (27). Infection might be a trigger for the autoimmune disease to develop. It could be that the patients with ITP have altered immune systems which makes them more susceptible to infection both before and after development of the autoimmune disease (28) .

Apart from the already acknowledged infections such as HIV and Hepatitis C causing secondary ITP, studies concerning infections prior to adult ITP-diagnosis are mainly case

studies reporting a possible association with candida and pneumonia (29, 30) . However, there are some studies regarding infection before autoimmune mediated diseases which describe the relationship between infection and disease as being multidirectional. The infection alone may not be enough to cause autoimmune diseases but some underlying predisposing mechanism makes the patients more susceptible to develop the autoimmune disease. An autoimmune disease can be triggered by miscellaneous infections, for example SLE can be triggered by Cytomegalovirus (CMV), Epstein Barr Virus (EBV), or Hepatitis C virus. It is the same for (RA), which that can be triggered by CMV, Hepatitis C, and other infections (24, 31, 32)

Previous studies have investigated and found that children tend to have infections before a diagnosis of ITP. In a prospective multinational study of children, infection was present in 56% of the patients before the diagnosis of ITP (33). In another study two thirds of those with ITP diagnosed had an infection some days to weeks before their ITP-diagnosis and in some a particular infectious agent could be directly identified, such as Varicella Zoster Virus (VZV), Epstein Barr Virus (EBV), Herpes Zoster (shingles) and upper respiratory infections. The characteristic of ITP in children differ from ITP in adults when it comes to severity of the disease and whether if it will become a chronic condition or not. Only 20% of children with low platelet counts and in contrast to the majority of the in adults diagnosed with the acute form of ITP will develop a chronic ITP (34). Infections before the onset of ITP in children are VZV, EBV, Herpes Zoster, upper respiratory infections, influenza and rubella and (34, 35). Mechanisms like molecular mimicry and cross-reactivity causing ITP from Helicobacter Pylori have been suggested and eradication of Helicobacter pylori have in some cases helped the patients. In the Swedish guidelines the ITP diagnosis includes investigation of Helicobacter Pylori (36) (37) (8).

1.7 Comorbidity
Primary immune thrombocytopenia is a burden to the patients not only regarding the symptoms of the disease and possible adverse effects of the treatment. The persistence of the disease can be lifelong and even though the risk of dying from symptoms of the disease is considered low, studies still show that the ITP-patients may have a shorter life expectancy then the general population; about a 2-fold higher mortality rate. Comorbidity with infection, cancer, cardiovascular disease and a more severe ITP disease increases the risk (38, 39). The immunosuppressive and cytotoxic drugs used for treatment of ITP along with an increased risk of comorbidity can possibly explain the increased risk of untimely

death. In a Danish study, where they compared ITP-patients with the general population, cause specific mortality in ITP-patients were 1.5 fold higher. The higher rates in the ITP-patients compared to the general population, ranging from 1.5-5.7, was suggested to be due to increased comorbidity with infection, cardiovascular disease, bleeding and haematological cancer (40). A speculation is that the immunosuppressive drugs and chemotoxic drugs can be involved and may even be the primary cause of untimely death in patients with ITP (38) . The increased prevalence of comorbidity, such as cardiovascular disease and haematological disease have been reported in other studies (41). The finding that the cITP-patients have higher mortality and not related to their risk of fatal bleeding makes it important to investigate the role of comorbidity and co-medication in more detail and if there is something to be done regarding early detection or treatment management in order to improve survival.

1.8 ITP and thrombosis

Platelets adhere and release activating mediators to release thrombin and the coagulation cascade begins. When a similar process occurs at an atherosclerotic plaque it can cause occlusive platelets and result in thrombosis. One of the mechanisms is that platelets are involved in the pro inflammatory mechanism promoting atherosclerosis. Platelet are involved in the immune defence as key mediators off inflammatory processes, releasing cytokines among other functions (4). In ITP the platelets are targeted by autoantibodies resulting in increased destruction furthermore there is decreased production. In the bone marrow the megakaryocytes are increased. Having thrombocytopenia can lead to symptoms of bleeding and an excessive amount of bleeding. Thrombosis is a deviation from the normal haemostasis that occurs after an injury and bleeding. Apart from the coagulation and platelet activation in thrombosis there is clot formation and vessel occlusion that can become life threatening (42). Patients with ITP have been found to have increased risk of thrombosis, a risk partly linked to their treatment especially to splenectomy (43, 44) even a long time after the splenectomy (45). The risk of thrombosis is also associated with antiphospholipid (46) (47) (48). Moreover, pro coagulant mechanisms in the platelets of patients with ITP compared to non-ITP seem to differ, such as increased plasminogen activator inhibitor-1, which makes the fibrinolysis resistant and possibly causing endothelial damage (49) along with other factors that are still unknown. In patients with VTE common risk factors for thrombosis and ITP related risk factors such as treatment and antiphospholipid are factors to be aware of. The low platelet counts and risk of bleeding in ITP does not protect against thrombosis (50). In the clinic it can be hard to balance between

protecting the patients from thrombosis and at the same time avoid symptoms of bleeding especially in patients with cardiovascular comorbidity. A score has recently been presented for clinicians for facilitating the care of these patients (51).

The median age of diagnosis of ITP is close to 60 years old. Many patients are likely to have comorbidity such as cardiovascular conditions e.g. hypertension and some may need to take antithrombotic and/or antiplatelet drugs which may affect their ITP disease and risk of bleeding.

2   ITP TREATMENT, CHAPTER 2

Corticosteroids is the first line treatment in severe or symptomatic ITP (6, 8) and this treatment has been used in ITP treatment since the 1950-ies (52). In case of severe thrombocytopenia requiring acute treatment where steroids are insufficient or contraindicated immunoglobulin infusions (IVIg) are given. This treatment is often effective in quickly improving the platelet counts. However, the duration of the effect is often short (2-3 weeks) and immunoglobulin may give rare but serious side effects like renal insufficiency(6) .

Platelet transfusion are used rarely as a temporary acute intervention in case of uncontrolled bleeding. Corticosteroids in lower doses are commonly used as maintenance therapy, sometimes during several years. However, there are some possible adverse effects of steroids and some patients do not tolerate them due to problems with sleeping, mania, gaining weight, high blood sugar or increase in blood pressure. The immunosuppressive function not only hinder the autoantibodies from attacking the platelets but moreover also lowers the immune defense with risk of infections. Bone density measurements are needed due to risk of osteoporosis when steroids are used for a long time (53, 54).

About 60-70% have a sustainable response in the form of safe platelet count after treatment with splenectomy (55). A major part of platelets and B-lymphocytes are pooled in the spleen and antibody bounded platelets are mainly sequestered by the macrophages in the spleen explaining the positive effect of splenectomy on the disease (56). The clinical challenge is that it is not possible to determine which patients will benefit from a splenectomy. The procedure is related to morbidity including chronic pain and porta vein thrombosis. Splenectomised patients are at increased risk for infections with pneumococci, Haemophilia influenza and meningococcal and need proper vaccination prior to operation (43, 57) .

Second line treatments other than splenectomy are Thrombopoietin receptor agonists (TPO-RA) and immunosuppressive agents like Rituximab (anti-B-lymphocyte antibody), Azathioprine, Cyclosporine, and cytostatics.

Rituximab is an anti-B-lymphocyte antibody that can be an effective second line treatment and in some patients have a long-term effect (58). However, it is not curative as splenectomy can be and has the negative effect of lowered immune defence months after treatment and is contraindicated in patients with Hepatitis B. There have been reports about rare events of progressive multifocal leukoencephalopathy when using Rituximab in ITP a couple of cases have been reported (6, 59).

Platelet transfusion is only indicated in the temporary treatment of serious bleeding manifestation as the effect of this treatment is very short. Symptomatic treatment of bleeding manifestations includes local treatment like in epistaxis and systemic treatment with Tranexamacid (a fibrinolysis inhibitor) that may help reducing skin mucous membrane bleedings.

The two available thrombopoietin receptor agonists (TPO-RA), Romiplostim and Eltrompopag, have mechanisms that increase the production of platelets through binding to the thrombopoietin receptor and have shown efficacy in raising platelets). In some patient's platelet counts may fluctuate severely making dose adjustment very troublesome. Adverse events include liver toxicity (eltrombopag) and an increased risk for thromboembolic manifestations (romiplostim and eltrombopag). Increased reticulin production in the bone marrow have been described in some cases but no clinical development to myeloproliferative disease have been reported to this date. Long-time follow up studies are ongoing. The thrombopoietin agonist function is a mechanism for increasing the cell proliferation, a mechanism that could theoretically lead to the uncontrolled cell proliferation and malignancies (2).

Treatment regimens. (60, 61)

➢ **First line**

Corticosteroids and immunoglobulin

➢ **Second line**

-Splenectomy

-Rituximab

-Thrombopoetinagnoists Eltrombopag or Romiplostim

-Immunosuppressive used rarely: Azathioprine, Cyclosporin A,

Cyclophosphamide, Danazol, Dapsone, Mycophenolate mofetil, Vance alcaloids

➢ **Refractory ITP**

Individualized, based on bleeding symptoms, age, activity level.

-TPO-RA (2, 8)

-Corticosteroids, intravenous gamma globulin, immunosuppressive agents,

chemotherapy.

Platelet transfusion (given at severe bleedings and surgery)(8)

3. MATERIALS AND METHODS, CHAPTER 3

Information from the Swedish Health Registers has been used to identify patients with ITP. Linkage has been possible using the unique personal identification number (PIN). In addition, we have included information from medical records in study II, collected from haematology and internal medicine units all over Sweden. In study III I had the opportunity to analyse data in the French database Système National des Données de Santé, (SNDS) where they are able to link data using the social security number, date of birth and sex.

3.1 DATA SOURCES

Swedish sources

3.1.1 The National Patient Register

The NPR was used to identify patients with codes D69.3 (immune thrombocytopenia) and D69.4 (other primary immune thrombocytopenia) from the tenth revision of the International Classification of Diseases (ICD-10) to identify adult patients (aged ≥18 years) with ITP. Those with two or more ITP diagnoses set at least 12 months apart (62) (63)were considered cITP.

NPR was founded in 1964 and from 1987 includes nation-wide data for all hospital health-care contacts covering details of hospitalizations such as date and duration of care, hospital/department name, surgical procedures and discharge-diagnoses with up to 21 contributory discharge codes (ICD, 7th–10th). The quality of the register has been shown to be of a high standard with almost complete coverage of inpatient care. Furthermore, since 2001 information on outpatient care has been registered and the coverage increased to a national level after a few years. The ICD-codes for different diseases were validated using medical records with a positive predictive value of 85-95% (62).

3.1.2 The Prescribed Drug Register

Information on prescribed drugs was obtained from the Swedish Prescribed Drug Register (PDR). The register has been available and linkable to other registers in Sweden since July 2005. PDR contains detailed information on filled prescriptions including product, quantity, dates of prescription and dispensing. The register has complete coverage and high validity (64). Drugs administered at hospitals are not recorded which is why we obtained this information from medical records in study two. Information about prescribed drugs was used to study infection before ITP diagnosis in study one and to study co-medication and served as a proxy for disease in study two. In study four the PDR is used to study co-

medication and as a proxy for some outcomes. Having both ICD10-codes for diagnosis and information about drugs makes the risk of misclassification of a disease less likely.

3.1.3 Total Population Register

The total population register contains information since 1968. The variables included are among others: PIN, date of birth, place of birth, emigration, divorce, marriage, county and residency. Furthermore, the register includes the multigenerational register with information about parents and siblings, the immigration register (including grounds for residence and reason to immigrate) and the PIN register (including PIN changes). From the total population register we retrieved information on emigration and death. We received comparison cohorts from the general population(65).

3.1.4 The Swedish Cancer Register

From the start of the cancer registry in 1958 it has been mandatory to report malignant and certain benign tumours to the register, 99% of them are morphologically verified. Regional cancer registries are since 1980: ies responsible of the registration helping the health care providers and making sure that the information is verified and making sure of a correct coding (66). From the cancer register we received information about primary solid and haematological cancer.

3.1.5 The Swedish Cause of death register

The cause of death register holds information about all causes of death since 1911. Electronic register based research information is available since 1952. Those who are Swedish citizens but died abroad are registered and since 2012 persons who die in Sweden but are not Swedish citizens are in the register. In the register there is information about residence, place of death, the date of death, underlying cause of death and a contributory cause of death (67).

3.1.6 The Longitudinal Integration database for health insurance and labour market studies (LISA)

The LISA register contains variables of socioeconomic (SES) character from 1990 in persons older than 15 years of age and from 2010 in patients older than 16 of age and can

be obtained from Statistics Sweden. Variables include highest attainable education, occupation, disposable income (including income for parental leave, retirement and unemployment) among other SES (68) (69).

3.1.7 Medical records

It is obligatory to enter ICD-codes for in and outpatient visits in the NPR and it is obligatory and regulated to keep a medical record for each patient (70). In the ethics section it is written more about ethics of using medical records for research purposes. The hallmark is that a medical record needs to be kept and stored in such a way that is maintained and safe from damage and protected in such a way that the secrecy of the patient is protected. Only health personnel who is actively involved in treating the patient can read it. With the patients consent an external person may be allowed to read it. For research purposes sometimes an exception to the rule of consent can be made if there is an ethical approval (see Ethical Considerations Chapter 4.) and the interest of the research is more significant than a possible violation of the patient's integrity.

From the medical records we could retrieve information not found in the PDR regarding both inpatient drugs and more detailed information about the prescribed drugs from the doctors including real world dates for start and stop dates. The medical records have information about when the patients didn't adhere or misunderstood the doctor's prescription. From medical records we collected information about platelet counts, leukocytes and haemoglobin and symptoms of bleedings. In comparison with the information in the NPR there were a lot more bleedings reported in the medical records, including milder bleedings where the patients specifically contacted the hospital because of symptoms of bleeding i.e. spontaneous hematoma and petechiae. In the NPR only serious bleedings were recorded.

The collected medical records are part of a longitudinal post authorisation safety study in which we are performing safety surveillance of a new drug. This thesis is independent academic research without participation of the pharma industry.

French sources

3.1.8 French database

The social security system in France started after the end of world war II, in 1945 (71), beginning in 1999 legislators proposed the construction of a system enabling health care

and drug utilization and gradually the national health insurance system (SNIIRAM) was created. The information was first registered for insurance purposes with information about in an outpatient visits along with dispensed drugs and drugs for chronic diseases collected for the French population (66 million inhabitants). The coverage is nationwide and includes employees in private sector, as well as public sectors, students and unemployed. The information is now a national health system database used in research with individual linkage data from 2009-2017 at this point.

-Systѐme National des Données de Santé, (SNDS) (72) is the new database from 2016 covering variables from SNIIRAM but also with the intent to grow and include information about cause of death and other parameters and also stores information from:

-The hospital database: Programme de médicalisation des systems des informations, with inpatient discharge diagnoses in all public and private hospitals in France (73);

-The inter-scheme consumption (Données de consommation inter-régimes - DCIR) that contains all outpatient reimbursed health expenditures, including community dispensing of reimbursed drugs, as well as chronic diseases allowing full reimbursement that are notified by general practitioners (74).

Diagnoses are coded using the International Classification of Diseases, version 10 (ICD-10). An anonymous identifier is derived from the social security number of insured individuals and can be used for linkage between the registers in the database. The in and out patient diagnoses are similarly coded with ICD-10 and procedure codes and dispensed drugs with ATC coding. In addition, the French database register chronic diseases in a certain register named ALD (Affections de Longue Durée), furthermore they register costly drugs given at hospital, which in Sweden is only possible to retrieve in medical records and in certain quality registers (72, 75).

4. ETHICAL CONSIDERATIONS, CHAPTER 4

The study was conducted in accordance to ethical review by the Regional Ethical Review Board, Stockholm (Record no 2009/4:10, addendums 2009 1597-31/4, 2009/1597-31/4, 2013/182-32, 2016/329-32, 2017/53-32 and 20181162-32).

The studies in this thesis are observational studies where no intervention is taking place. The patients are identified in registries by the Swedish National Board of Health and Welfare and each patient's identification number is replaced with a serial number to ensure anonymity of individuals. Results are published on aggregated level to prohibit backward identification of individual patients. There is a possible violation of integrity when taking

part of information from registries and especially when reading the patients' medical records. However, once the data is extracted from the medical record the recording is done by using a code which is provided by the National Board of Health and Welfare. An exception to having the patients consent can be made for research purposes if the benefit to the entire patient group or population is regarded as greater than the potential integrity violation in reading a patient's medical record. In studies involving human's ethical considerations needs to be taken into account and an approved ethical application is required. In observational studies where only health registers are used we expect the potential violation of integrity to be small. The violation of integrity of taking part of the patient's information must be weighed against the benefit to the patients with the disease and potentially to the general population.

The ethical approval is required to perform a study and to be able to apply for data. The strongest ethical consideration in my thesis is in our study II where we included medical records to have more details that we could not find in the registers e.g. lab values. We did not ask for the patient's permission since we identified the patients in the register and we could not be sure that the diagnosis code was correct. There is a risk that the worry would have been a larger problem than the potential violation of integrity when we read their medical records. Only the research group took part of the information and we were only observing retrospectively without any attempt to affect treatment or else. According to the Swedish Personuppgiftslagen (1998:204)19§, which in May 2018 was replaced by GDPR, it was stated that sensitive information can be used for research purposes without informal consent from the patients, if the study is approved by an ethical board and only if the treatment is necessary and/or the research is necessary and has more bearing than the risk of violation of integrity of the patients (76). According to § 20-22, since January 2019, if an ethical application is approved, research without consent can be allowed if the study participants will not be harmed and the benefit to the patient group is of higher benefit than any potential violation of the participants integrity (77) (78).

France

The data in France was analysed with the accordance of the Institut des Données de Santé in March 2012 (no. 40) and the Commission Nationale de l'Informatique et des Libertés in July 2012 (no. DE-2012-076). In the database three and nine variables respectively are used to link the different data bases together, only the research group who have applied for access to the data and have both ethical approval and approval to work with the data are allowed to do so.

5. EPIDEMIOLOGY IN HEALTH SCIENCE, CHAPTER 5

5. 1 Epidemiology in health science

The word epidemiology is an ancient word from the Greek words epi (on or upon) demos (the common people) and logy (study) (79). In short, the objectives of epidemiology can be characterized as:

-study the natural course of a disease

-determine the incidence and prevalence of disease in a population

-identify patterns and trends of disease occurrence

-find the aetiology/aetiologies of a disease

-study the effect and safety of disease prevention and treatment (79).

In epidemiology incidence and prevalence of disease can be investigated and whether or not there is a connection between an exposure and an outcome. One of the first epidemiologist was doctor John Snow who managed to find out he exposure behind the cholera outbreak in London in 1854. He compared cholera mortality between people who got water from different sources. Snow found that these people were alike in all other aspects except from getting water from different pumps. The factor that differentiated the people who got cholera from those who didn't was that the water passing through London was seeded. This is a good example of how looking at a larger picture and comparing people to each other in order to find out what can be the cause of an outcome can help identify a problem, however this was rather a natural experiment where the participants were not randomly assigned to the pumps which would be the case in a clinical trial (80).

5.2 Pharmacoepidemiology

 Pharmacoepidemiology has had a status as a specific discipline since the mid-1980:ies (81). The focus is on the efficacy and safety of drugs (74, 82, 83). Observational pharmacoepidemiological studies enables evaluating a more heterogeneous group of patients than what is usually included in randomized controlled trials. The benefit of observational studies is that they are more suitable for post authorization studies and long-term follow up and allow for larger cohorts. The disadvantage is that the randomization in clinical trials is harder to replicate.

In order to study drug safety in patients it is useful to perform an observational study in order to find out questions like:

-the adverse event is in fact an adverse event and not disease progression

-if there is interaction with other drugs

-if some patients may be more susceptible to certain adverse events, the events may be associated with age, sex, a comorbidity i.e. liver disease or an underlying cardiovascular condition (84).

We strive at comparing patients with similar baseline variables considering age, sex, comorbidity and other factor that can have an impact on the outcome.

 With permission from photographer Ulrika Undén.

6 STUDY DESIGN AND STATISTICAL METHODS, CHAPTER 6

6.1 Study design

In observational studies a cohort study can be the design of choice when one wants to examine risk factors for a certain event or several events. The study participants are classified as either exposed or unexposed and can be followed to see if an event (or several) events occur (85). A closed cohort can be used if we set the cohort from the beginning, an open cohort if there is need for a continuous inclusion of study participants. The study cohort is followed until the time of censor, either the event of interest occurs or one of the most common censor variables: death, emigration or end of follow up. All the studies in this thesis are cohort studies, in study IV we performed a matched cohort study.

The case-control can be efficient when studying a rare disease and ITP is a rare disease. However, we use nationwide data cohort studies, suitable for rare disease and rare outcomes (79).

6.2 Statistical methods in this thesis

Standardized Incidence Ratio (SIR) was used in Study I to calculate the ratio between the observed rate in the patients with cITP and the rate in the general population. The hypothesis is that the patients with cITP have a higher rate of infections than the general population. Considering that age and sex are likely to influence the risk of infection we calculated the Standardized Incidence Ratio, comparing patients with ITP to the population by age and sex. The Standardized Incidence Ratio method is a straight forward way to compare what we expected (the calculated rate in the population) to what we observed in the patients with ITP and to evaluate the difference by looking at the ratio (86).

In study II, we described a cohort of cITP patient's characteristics at treatment start. A multinomial logistic regression analysis was used. In multinomial logistic regression we can analyse the effect of each independent variable (covariates such as platelet counts, bleedings, etc.) on each category of the dependent variable (start of treatment with corticosteroids in one category and start of other ITP treatment in the other) compared to the reference category, in this case no treatment (87). The method allowed us to compare two treatment types to no treatment start in the same model and at the same time include several factors and thereby adjusting for several covariates that may have an impact on treatment start. Regression is often used when the objective is to study causation, in observational studies this is rather an association (88).
Moreover, a t-test was used to compare the mean time to splenectomy between two time

periods by calculating the probability of the observed or a more extreme difference under the null hypothesis of no difference (p-value). A t-test is a way to see if there is a statistically significant difference between groups (88).

In study III we performed a cohort study using survival analysis and adjusted for age, sex and other relevant covariates (89). In our main analysis we performed a time dependent multivariable Cox counting process (90) to estimate associations between risk factors of Arterial Thrombosis and Venous Thromboembolism, respectively. All identified confounding variables such as age, sex and comorbidities were adjusted for, including time-varying covariates and fixed covariates. For French and Swedish data, we spent a lot of time harmonizing the protocol to make sure that the same variables were included, regarding the use of ICD and ATC codes. Furthermore, we verified in what way the diagnoses are decided on in order to have the same severity of disease. For the outcomes, we restricted to in hospital diagnoses of arterial thrombosis and venous thromboembolism to capture only severe outcomes.

A matched cohort study with 10 comparators from the general population per patient with ITP was performed in study IV. To eliminate some factors that could have biased the results we matched the patients with ITP to the population by age, sex, calendar year and county. Further adjustments were performed regarding comorbidities, number of hospital visits as a measure of frailty and socioeconomics (education and income). We performed a COX analysis with different time intervals, one year after index, 2-9 years after index and 10-20 years after index.

7. METHODOLOGICAL ISSUES, CHAPTER 7

Internal validity

A study should aim for having a high internal validity, meaning that measures have been taken to have the best possible quality of the study performance. This means starting with a thorough literature search, writing a good study plan, choose an appropriate design and carefully consider how to deal with missing values, classification, control for confounding, selection bias, information bias and other methodological issues.

External validity

Generalizability of a study, if the results from the study can be applied in another setting, is connected to the external validity. Data from more than one setting can increase the external validity of the study.

Random or systematic errors

Bias is a systematic error that causes an incorrect (over or under) estimate of an association. In observational studies it is important to consider sources of bias and how to deal with them.

Selection bias-If the selection of controls in a study is not randomly sampled nor representative of the study base selection bias can be introduced. The comparison between cases and controls may then be biased in the analysis.

Information bias- a bias related to measurement errors, a misclassification.
Non-differential misclassification is a random information bias when we have no reason to believe that the bias effects one group more than another, there is bias due to misclassification but exposed and non-exposed are probably affected in the same way. The association will be affected towards the null.

Differential misclassification is a non-random information bias when the information is better in one group than another, which can result in an over or underestimation of the association.
Surveillance bias can be present if a group of patients who are followed in a hospital setting are compared to a group that are not. The possibility of finding a disease in these patients is more likely since the patients are already followed in a hospital setting. There is risk of detection bias due to the surveillance of the patients.

Reverse causation (Protopathic bias)-The exposure is affected by an underdiagnosed occurrence of the outcome as an underlying cause. The exposure-disease process is reversed.

Confounding-a confounder is a variable that affect both the exposure and the outcome. In observational studies confounding is always something to consider and aim to control for. This can be done at the design stage, such as matching patients with controls. Other tools are by restricting e.g. include only women in a study if sex is a confounder. Restricting can in some cases introduce bias if the excluded patients or variables have characteristics that impact the results (91). In clinical trials randomization is a common way of dealing with confounding. At the analysis stage stratification is the most common tool for confounder control in cohort studies. A disadvantage of stratification is that we cannot control several variables at the same time and moreover there will be fewer persons in each strata and when we analyse the data the power in each group is reduced.

Type I Error

A significance level of 0.05% is used more often than 0.01% level in observational studies. The significance level means that there is a 5% chance of rejecting the null hypothesis even though it is true, in other words to commit a type I or alpha error.

Type II error

If we fail to reject the null hypothesis even though it is not true we commit a type II or beta error. The statistical power is a measure of how well the null hypothesis is correctly rejected when in fact the alternative hypothesis is true. Significant testing needs to be interpreted with caution (92), a statistically significant result is not always the same as being clinically relevant (80, 85, 93).

Reflection on bias in the studies included in this thesis:

In study I we considered confounding by age and sex and adjusted for these variables in the analysis. We used national registers and had little concern for selection bias. Considering that data came from the same source we expect potential measurement errors to be non-differential. Infections were measured before diagnosis of ITP, but there was a risk of detection bias regarding the diagnosis of ITP. In the sensitivity analysis we considered misclassification due to mild ITP and included only those exposed to ITP drugs, this did not change the results. We also considered the fact that the patients may have started ITP treatment prior to the time when the diagnosis code is set in the register.

Therefore, all who had immunosuppressive treatment 30 days before infection or infection drug were disregarded. This did not change the results either.

In study II the risk of misclassification is limited since every medical record is manually evaluated before it is classified as cITP. The information in the database where the information from the medical record is entered is quality controlled and entering errors checked and changed. However, there is risk for missing information, only those who are still followed in a hospital setting can be included. There may be loss to follow up for patients with a mild ITP disease who are discharged and go on to have their visits in primary care. Considering our aim to study start of treatment this should not be a major concern since the patient with chronic ITP are diagnosed in outpatient or inpatient care and require at least one year of follow up to have a cITP diagnosis. Possible predictors of treatment were included in the analysis and adjusted for.

In study III There are some unmeasured risk factors such as information about platelet counts, genetic risk factors, smoking, immobilization and exposure to ITP drugs. We were aware of the risk of misclassification and checked the variables we did include carefully to make sure they had the same connotation in order to limit the risk of information bias. We adjusted for potential confounding such as age, sex and co-medication in the analysis. The risk of selection bias should be limited since we included national databases

In study IV In order to maintain proportional hazards and because of potential detection bias when we compare patients with ITP to the general population we used time intervals in the analysis. We matched the comparison cohort to be similar to our patients with ITP in the study design, and specified other variables in the model, such as comorbidity, that could bias the outcome. Residual confounding remains, we lack certain information in the register such as smoking. Information about both patients and matched comparison cohort came from the same registers and the bias should be non-differential.

8. SUMMARY OF ALL STUDIES. CHAPTER 8

8. 1 Study I

☐Research question: The aetiology behind primary ITP is unknown, causes of secondary ITP include other diseases and virus e.g. HIV. Acute thrombocytopenia can follow an infection. There are several possible mechanisms to why an infection can be involved in the loss of tolerance mechanisms that develop in autoimmune disease. Furthermore, the relationship can be bidirectional meaning that the patients with autoimmune disease have more difficulty to battle infection. We wanted to investigate if the patients with ITP had been exposed to more infections before their disease compared with the general population. Since the incidence of chronic ITP has not been determined after the new definition of phases of the disease in 2009 we wanted to find out the incidence according to the new definition.

☐Materials and methods: Information about diagnosis of infection and anti-infective drugs was obtained from the Swedish Health Registers. Patients with cITP were included and were used to compare the rates of infections between the cITP-patients and the general population. The research question was approached using standardized incidence ratios (SIR).

☐Statistical analyses: Standardized Incidence Ratio adjusted for age, sex and calendar year, was calculated to compare the rate of infection between patients with ITP to the rate in the general population. The expected number of events was calculated by first adding all person-years accumulated in the cohort from the general population, divided into strata defined by sex, age (in five-year groups) and calendar year of observation (in 1-year intervals). Each stratum specific person-time was then multiplied by the corresponding stratum-specific prevalence rate obtained from the entire Swedish population. The 95 % confidence intervals (CI) were calculated assuming that the observed events followed a Poisson distribution. Some patients may have been treated with immunosuppressant due to thrombocytopenia before the ITP-diagnosis was recorded in the register. To minimize the risk of misclassification and rule out that the infection was due to the immunosuppressant drug a sensitivity analysis was performed where all infections and anti-infective treatment that were recorded within 30 days after an immunosuppressant therapy were disregarded. As an additional sensitivity analysis, we included only those patients with cITP who received ITP-treatment in order to exclude those with an ITP at subclinical level. SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA) was used to analyse the data.

Results and discussion

Main results (all results in paper I page 809-811).

Time period	2009-2014
Number of cITP patients:	1087
Female	556 (51%)
Time period	2009-2014
The incidence of patients with cITP	2.3 (2.15-2.45) per 100 000
Mean age at cITP diagnosis, years	57
Female:	54 (SD 21)
Male:	61(SD 20)
All infections, diagnosis	Observed 167 Expected 19 SIR 8.7 (7.4-10.1)
Female	SIR 11.1(8.8-13.9)
Male	SIR 7.36 (5.9-9.05)
All anti-infective drugs	Observed 474 Expected 347 SIR 1.4 (1.3-1.5)
Antibacterial drugs or systematic use	Observed 435 Expected SIR 1.3 (1.2-1.5)

Discussion: The patients with primary cITP had an increased rate of infections within five years before diagnosis of ITP compared with the general population of the same age and sex. Considering the bidirectional relationship between autoimmune disease and infection this can have a biological explanation. In autoimmune disease the dysregulation of the immune system makes it more difficult to take care of pathogens (28). An association between infection and other autoimmune disease such as autoimmune thyroid disease and systematic lupus erythematosus have been reported (31, 94). This is important knowledge considering the aetiology of ITP is still unknown and because treatment for ITP is immunosuppressive or immunomodulatory. A limitation to the study is that we did not adjust for confounders other than age and sex, comorbidity might have an impact. However, the difference was quite large and if we assume that the bias from comorbidity is non-differential the higher ratio of infection needs to be considered. ITP is a diagnosis of

exclusion and sometimes the patients receive ITP-treatment before the diagnosis code is registered. A sensitivity analysis was performed to exclude those with an anti-infective treatment a month before ITP. An additional sensitivity analysis was performed to include only more severe ITP patients who received ITP-treatment. The results did not change in these analysis.

8.2 Study II

□Research question: There are no guidelines only recommendations for treating patients with ITP and several articles point out that evidence based studies are warrant. We were interested to perform a study on real-world data to see the clinical practice of ITP treatment. The hypothesis was that the patients are treated to avoid symptoms rather than receiving prophylactic treatment and maintain a normal platelet count. We were interested in how patient characteristics influence start of treatment.

□Materials and methods: Adult patients with chronic ITP from 2009-2016. National Health Registers and medical records.

□Statistical analyses: Characteristics of the study participants including time since first ITP diagnosis to start of treatment, platelet count during 10 days preceding start of treatment, comorbidities during 90 days preceding start of treatment, co-medications during 90 days preceding start of treatment and duration of treatment were shown using descriptive statistics such as numbers, mean, median and proportions. Frequency distributions for categorical variables were generated.

We predicted treatment start by using a multinomial logistic regression to calculate the odds ratio for the different patient characteristics. We investigated the same factors in the model as in the descriptive analysis. The multinomial logistic regression allowed us to have two outcomes in the model with two different treatments (corticosteroids and other ITP treatments)and no treatment start as reference.

□Main Results. All results in paper II: In a cohort of 858 patients with cITP the patients' start treatment at relatively low platelet counts, median platelet counts $12*10^9$/L. Severe bleedings are present in 75 (13%) at treatment start. Co-medication influence treatment start, patients with diabetes were less likely to start treatment with corticosteroids and patients with antihypertensive drugs had higher odds of starting treatment. Corticosteroids are the most common treatment in 537(92%) of the patients. In figure 3 the distribution of therapeutic drugs and splenectomy after initial corticosteroid treatment is shown.
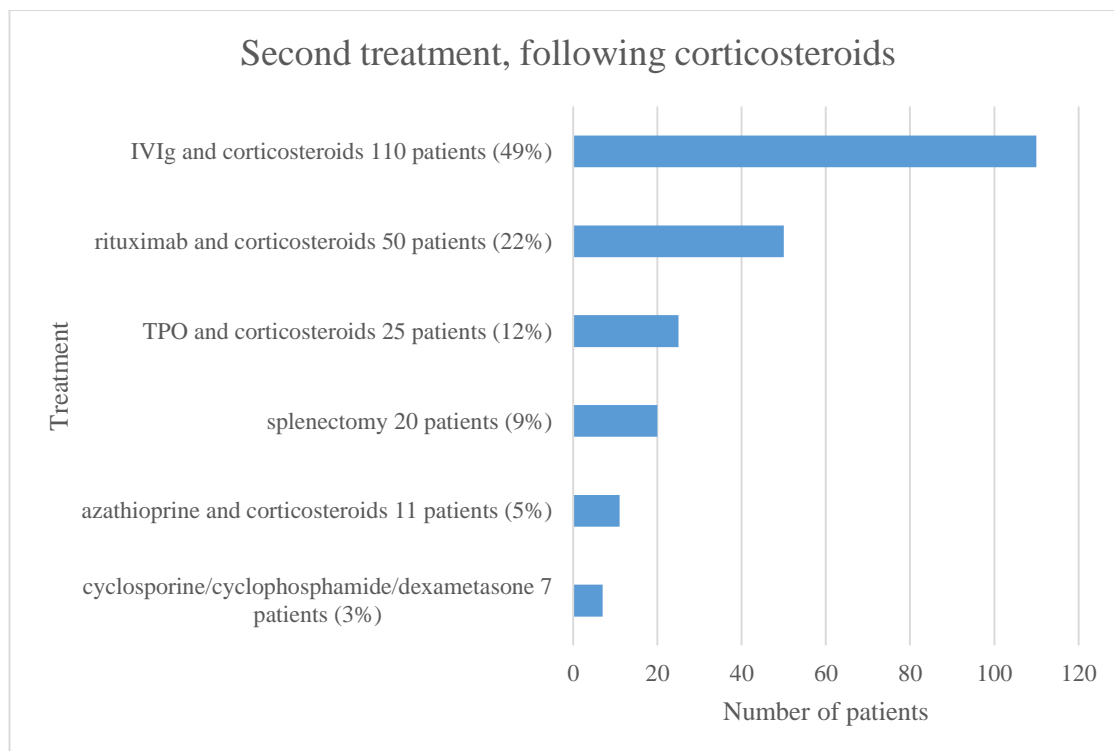
Second treatment, following corticosteroids

Figure 3. Second treatment given after corticosteroids.

Discussion: Our study gives real-world evidence study to the recommendations to treat in order to avoid symptoms rather than aiming for a normal platelet count, and avoid unnecessary treatment. Among the patients with severe bleedings a majority of them had comedication with antithrombotics and antiplatelets. Previous studies have found platelet counts in line with ours (95) (96)and an increased risk of bleeding in patients above 80 years of age (97). Including both medical records and information from the register helped us answer the research question about characteristics at start of treatment and the impact from comorbidities (i.e. diabetes) on treatment start. It was especially important to have information on platelet counts and milder bleedings such as hematoma and epistaxis which is rarely reported in the NPR but remains problematic symptoms for the patients. We had a good representation of hospitals from smaller to large university hospitals but lacked information from primary care. However, the diagnosis is generally from a hospital setting and we focused on treatment start in incident patients.

8.3 Study III

Research question: Our hypothesis was that not only rate of venous thromboembolism moreover the rate of arterial thrombosis is increased in patients with ITP and we were interested to know if and if certain risk factors other than age, sex, comorbidities and co medication might have an impact on the rate.

Materials and methods: Two nationwide registers, similar ICD-codes and ATC-system in both countries investigate baseline covariates and risk of AT and VTE in patients with primary ITP of all stages.

Statistical analyses: We included all patients identified with an ITP diagnosis during the study period and estimated incidence rates of AT and VTE, first in the overall ITP population in each country, and then by baseline risk factors. Patients with the risk factor at baseline were compared with patients who did not have the risk factor. We performed a cohort study using a COX survival analysis and adjusted for age, sex and other relevant covariates (14). In addition, we performed a sensitivity analysis in which we adjusted for age and sex. In our main analysis, we performed a time dependent Cox counting process where all identified confounding variables were adjusted for, including both time-varying covariates and fixed covariates. In the Cox counting process the data is split in different time intervals and all covariates are taking into account in the analysis.

- Main results, all results in paper III pages 126-129:

Arterial Thrombosis

ARTERIAL THROMBOSIS	SWEDEN	FRANCE
Number of patients	2490	7225
Incidence of AT. with 95% CI	14.7 (12.4-17.5)	15.0 (13.4-16.7)
Multivariable analysis, HR with CI 95%		
Sex, ref male	0.7 (0.5-1.0)	0.5 (0.4-0.7)
Age groups, 18-39	Ref	Ref
40-59	5.8 (1.2-28.5)	8.4 (3.2-22.2)
60-74	25.1 (6.0-105.0)	19.7 (7.5-51.3)
>=75	75.4 (18.1-314.4)	18.6 (7.1-48.7)
History of AT	3.1 (2.0-5.0)	2.5 (1.8-3.5)
Antiplatelet drugs	1.4 (0.6-3.2)	1.6 (1.2-2.1)
Kidney disease	2.6 (1.2-6.0)	1.2 (0.9-1.7)

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Venus thromboembolism

VENOUS THROMBOEMBOLISM	SWEDEN	FRANCE
Incidence of VTE, with 95 % CI	6.5 (5.1-8.4)	6.9 (5.9–8.1)
Multivariable analysis, HR with 95% CI		
Sex, ref male	0.9 (0.5-1.6)	1.2 (0.9-1.7)
Age groups, 18-39	Ref	Ref
40-59	2.5(0.6-10.4)	1.9 (1.1-3.4)
60-74	5.6 (1.6-19.8)	2.3 (1.3-4.0)
>=75	15.0(4.2-54.0)	3.5 (2.1-6.0)
History of VTE	13.5 (5.5-33.4)	3.5 (0.8-14.2)
Cancer	0.6 (0.2-2.0)	1.8 (1.2-2.7)

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Discussion: The finding of similar rates of arterial thrombosis and venous thromboembolism in France and Sweden strengthen the finding of a higher rate compared with a general population. Previous studies report that thrombocytopenia does not mean a lower risk of thrombosis (38, 98). The finding is important considering the median age of ITP is almost 60 years of age, the patients are expected to have comorbidities. Co-medication with antiplatelet and antithrombotic drugs indicate an underlying cardiovascular disease and is an additional risk factor for thrombosis in the patients with ITP. Comorbidities are important to take into consideration when evaluating the risk of bleeding and thrombosis and a score for helping clinicians had been proposed (51). A high index of

suspicion of both arterial thrombosis and venous thromboembolism is warrant in treating these patients, while carefully evaluating their risk of bleeding. In a future study it would be of interest to include ITP treatment. Considering IVIg, romiplostim and rituximab are given at the hospital and not included in the Swedish Prescribed Drug register (available in the French registers) this would require medical records.

8.4 Study IV

Research question: An increased risk of haematological malignancies have been associated with ITP in previous studies. Solid tumours are not so investigated apart from a few studies where stomach cancer has shown contradicting results. There are molecular mechanisms involving the loss of tolerance that may be involved in a less strong way to attack tumour antigens that can lead to the suspicion of an increased risk of tumour development in patients with autoimmune disease.

The NPR was used to identify comorbidities and the Charlson comorbidity index calculated. Moreover, amount of in and out hospital visits was defined. The general population comparison cohort was randomly selected by the TPR and matched on age, sex and calendar year.

Materials and methods: Matched-cohort using the Swedish National Health registers to compare adult patients with ITP of all stages (newly diagnosed, persistent and chronic) to controls from the general population matched on age, sex, county and calendar period in study period 1997-2016.

Statistical analyses: First we calculated incidence rates and incidence rate ratios between the patients with ITP and the matched cohort. We compared the impact by covariate on each cancer outcome. In the main analysis we investigated if the patients with ITP have higher rates of overall and organ specific cancers compared with the matched comparison cohort from the general population. Cox proportional hazards regression was used to estimate the risks. The underlying time scale was the follow-up time from diagnosis of ITP or index date for the comparison cohort. We used time split in three categories first year, 2-9 years and 10-20 years in order to keep proportional hazard in the model and to be able to identify possible detection bias. A comparison cohort from the general population were matched by sex, age groups, socioeconomic index and residential place.
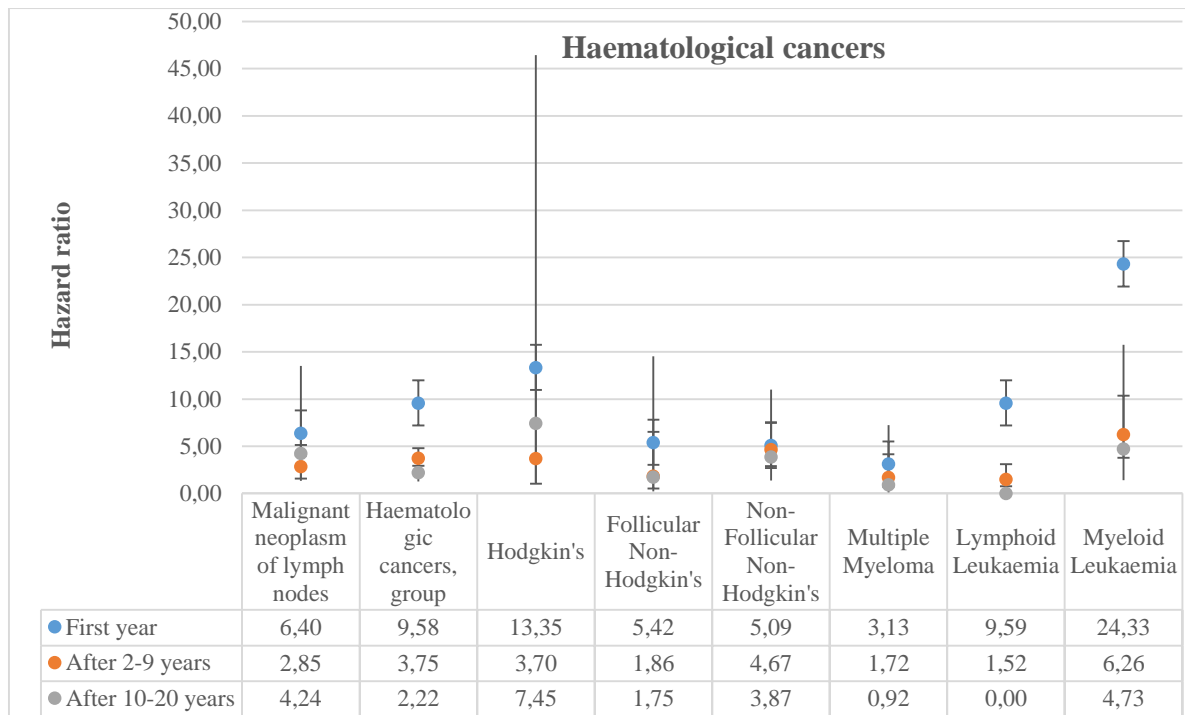
Figure 4. Hazard Ratios (HR) for cancer in patients with ITP compared with the matched cohort in time intervals. Adjusted for age, sex, residential place, index year, Charlson comordity index score, number of hospitalizations and outpatient care visits, income, education.

Results: The mean follow-up time after diagnosis of ITP was seven years (standard deviation 5.1. The number of patients with primary ITP were 6 740 (55% women) and 59 394 (55% women) persons in the matched comparison cohort. The incidence rate ratio (IRR) of overall cancer in patients with ITP was 1.45 (95% CI 1.35-1.56). The main results of the study are presented in Figure 4 and 5. The hazard ratios at different time intervals (first year, after 2-9 years and after 10-20 years) for haematological cancers are presented in Figure 4. In Figure 5 hazard ratios for any cancer, cancer in the digestive organs as well as site-specific cancers are presented in time-intervals.
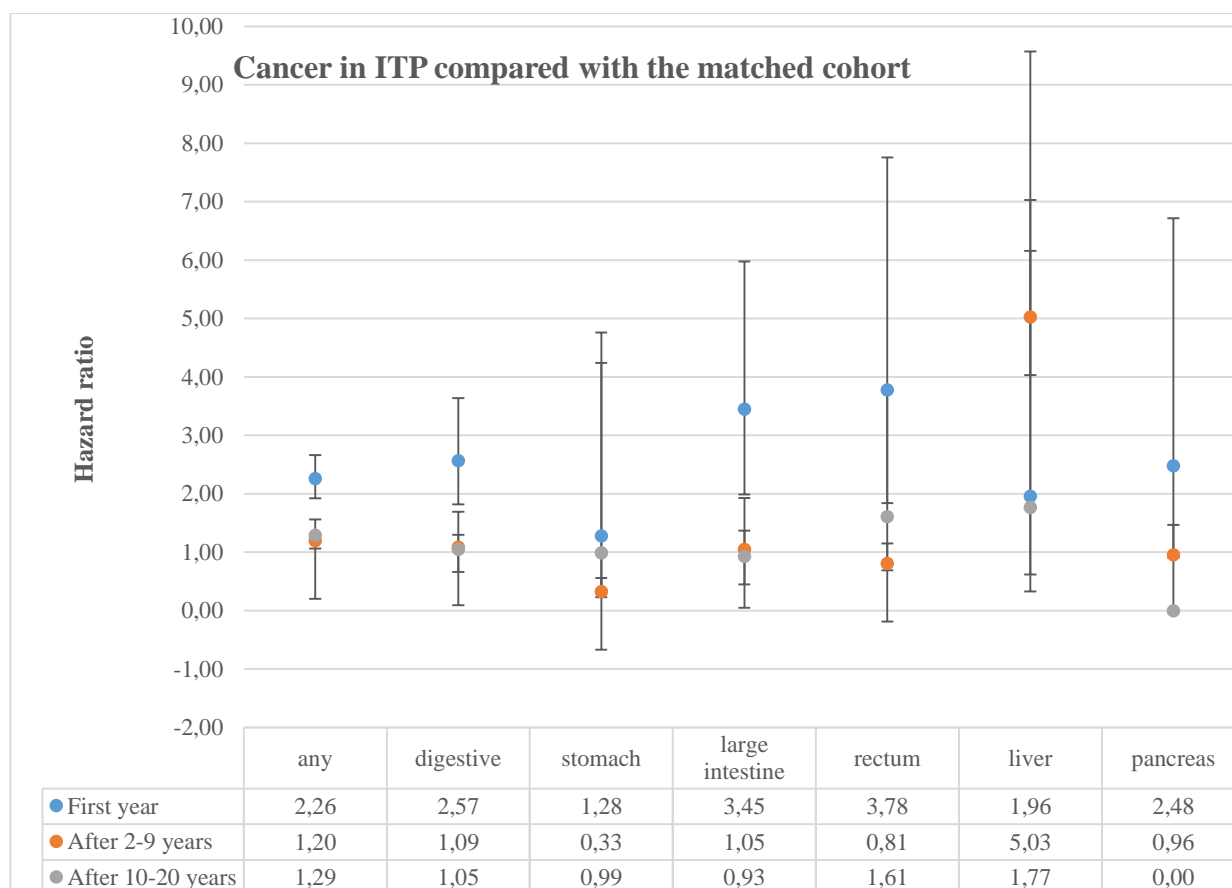
Figure 5. Hazard Ratios (HR) for digestive tract cancer in patients with ITP compared with the matched cohort in time intervals. Adjusted for age, sex, residential place, index year, Charlson comordity index score, number of hospitalizations and outpatient care visits, income, education.

Discussion: We confirmed an increased risk of haematological malignancy in patients with ITP compared to the general population (99-102). The risk is important knowledge regarding the surveillance of the patients. Some patients with ITP have a mild disease and are discharged from follow up since their thrombocytopenia is regarded mild. Is the risk of haematology malignancies related to severity of the ITP disease? Future studies are warrant; it could be of importance to see whether or not all patients with ITP may benefit from continuing their surveillance considering the increased risk of hematologic malignancies.

The graph above shows that the increased risk for most cancer diagnosis is more pronounced the year following diagnosis of ITP, liver cancer is more pronounced after 2-9 years. After adjusting for sex, age, socioeconomics, comorbidities and number of hospital visits we found an increased risk of solid tumours in patients with ITP compared to the general population, more pronounced in men. Certain cancers (ovarian, brain and large intestine) were only increased during the first year following diagnosis of ITP which can be considered as a probable detection bias. The patients are thoroughly investigated due to their ITP and early symptoms and abnormal lab values indicating cancer can be detected. Liver cancer and skin cancer were still increased years after diagnosis which is more of a

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signal of an increased risk in these patients. The result is of importance regarding surveillance of these patients and when developing and evaluating new drugs. When we interpret the result of an increased risk in liver cancer in males with ITP we tried at first to adjust for alcohol use. There were too few registered events of alcohol dependency and liver disease due to alcohol in the register to be able to adjust for it, however alcohol related thrombocytopenia is defined as secondary ITP and when the primary ITP diagnosis is given excessive alcohol drinking is usually ruled out. There are certain activity of destruction of platelets by antibodies not only in the spleen but also in the liver that separates the patients with ITP from non-ITP (103), the mechanisms in relation to cancer needs more evaluation.

9. DISCUSSION, CHAPTER 9

9.1 Strengths

The observational studies performed in this thesis have the advantages of limited selection bias, long follow up and no recall bias. The strengths of these studies include the population based design and the possibility to include register data for all patients with primary ITP during the study period. This means there is limited risk of selection bias of a certain group with particular characteristics. We obtained information on both inpatients and outpatients, to identify all hospital contacts during the study period. The accuracy of diagnoses recorded in the Swedish Patient Register is generally high (62). Moreover, we used data from the PDR which provides information on all dispensed pharmacological treatments. In study I and IV the comparison group is the Swedish general population, in study IV ten matched controls from Statistics Sweden, limiting the selection bias. However unmeasured confounders can never be completely ruled out.

In addition, in study II we have medical records which contains detailed information not found in registers including blood values and treatment received at the hospital increasing the internal validity of the study when such detailed information can be used. Furthermore, I am very familiar with the data after 10 years of abstracting medical records from patients with ITP. Every year the data is quality controlled for data entry mistakes which the data abstractors correct and if we lack data we collect it continuously, ensuring a high quality of the collected data from the medical records. Even with national registers Sweden is a small country when studying details about a rare disease. Even though we didn't pool the data in study III the findings in our study is strengthened with the data from another country and with the similar results. In particular, such a large country as France with 66 million people included in a linkable database. Furthermore. the fact that both countries had the same variables and that we used the same analysis in both databases, increased the internal validity.

9.2 Limitations

The current studies have some limitations. The lack of primary care data is a limitation because patients may have follow up visits in primary care and we lack access to this information. We have information from the PDR on all pharmacological treatments prescribed from primary care that are dispensed. But there is no indication for treatment and for the studies with chronic ITP we lack medical records from primary care, so there is risk of loss to follow up for those patients, most probably when the cITP disease is milder. We also lack information from cITP patients who are only treated for their ITP at another clinic i.e. obstetrics. There might be more patients with mild ITP who never seek medical care even though they might have thrombocytopenia. In common with other registry studies from Sweden (apart from certain quality registers) we lack information about drugs given at hospital. Fortunately, we had collected medical records and were able to use the information to analyse start of treatment and treatment pattern in study II.

9.3 Conclusion: Findings and implications

I. In study I we reported the incidence of cITP and an increased rate of several infectious diseases prior to the diagnosis of ITP. This has implications considering that patients with ITP are often treated with immunomodulatory treatment and the finding may be a mechanism in the dysregulated immune system of autoimmune patients. We did not find a new cause of a secondary ITP but contributed to the scientific evidence that there is an association between infection and ITP.

II. In study II we were interested to describe the patients with ITP more in detail and learn about their treatment pattern and start of treatment since previous studies had questioned how severe their symptoms were in relation to treatment. We observed that their treatment started at low platelets and that co-medication such as antihypertensive drugs and diabetes influenced the treatment decisions. Platelet counts below $20*10^9$/L, antihypertensive treatment and bleedings were the strongest predictors of treatment start and diabetes yielded lower odds to start corticosteroid treatment. In the latter years' splenectomy is less common and time to splenectomy delayed. There was a call for evidence based studies in this field. We contributed with a scientific investigation with detailed information from both registries and

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medical records about characteristics of treatment start, which can be informative for clinicians treating patients with ITP.

III. In study III we investigated the impact of comorbidity on the rate of AT and VTE in patients with ITP in two national databases We found that age and male sex are the most important risk factors for AT and age for VTE. Baseline risk factors increase the risk later on. When treating patients with ITP, the occurrence of risk factors associated with the disease and with thrombosis, should be acknowledged. The results from two nationwide studies have similar results regarding the rate of AT and VTE, giving evidence to the importance of being aware of an increased risk of AT and VTE which of course is important for clinicians, especially in an elderly population with comorbidities.

IV. Risk of cancer in patients with ITP. We can confirm that the risk of hematological malignancy is increased in patients with ITP compared with the general population. The results from our investigation of the most organ-specific cancers revealed an increased risk of liver cancer and skin cancer in ITP compared to the general ppopulation several years after diagnosis of ITP.

❖ Summary of the main findings and implications in this thesis:

In this thesis incidence of patients with primary chronic ITP in Sweden were reported. The main findings are that even though the ITP disease seems quite mild in terms of symptoms of severe bleedings and we learnt that asymptomatic patients can fact be left untreated there are other factors complicating the disease outcome. We found that the patients with ITP have a susceptibility to infections prior to the diagnosis of ITP and an increased risk for both arterial and venous thromboembolisms. We conclude that clinicians need to have a high index of suspicion for cancer (haematological malignancies, liver and skin cancer in particular), risk of infections and thrombosis in patients with ITP.

9.4 Future perspectives

➢ The incraesed risk of cancer needs to be be further investigated with respect to severity of ITP disease and exposure to ITP drugs.

➢ There is need for more knowledge about how patients with ITP are affected in their everyday life, by the disease itself and due to the treatment. There are previous

studies reporting that patients with ITP have problems with fatigue and that they are staying away from social activities. A survey study should be performed. At the same time information about experience of adverse-events, especially milder adverse events of drugs could be collected. These kind of studies are lacking.

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