Disease pathogenesis, treatment effectiveness, and co-morbid burden among adult patients with primary immune thrombocytopenia (ITP)

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Summary

Background

Primary immune thrombocytopenia (ITP) is an autoimmune disease involving autoantibody-mediated platelet destruction, suboptimal platelet production, and T-cell-mediated platelet lysis. These processes cause a decreased peripheral blood platelet count (< 150 × 10^9/L), resulting in an increased susceptibility to bleeding events. While the course of primary ITP is often acute (< 6 months) among children, it is generally chronic in adults. Despite a marked increase in epidemiological research over the past decade, unresolved questions remain with regard to disease pathogenesis, treatment effectiveness, and co-morbid burden among adults with primary ITP.

Objectives

1. To launch a registry for adults with primary ITP in the United Kingdom (UK)
2. To evaluate associations of candidate single nucleotide polymorphisms (SNPs) with primary ITP
3. To assess the effectiveness of ^{111}In-labelled platelet studies in predicting outcomes from splenectomy
4. To document health-related lifestyle concerns among patients
5. To gauge the effectiveness of *Helicobacter pylori* (*H. pylori*) eradication in elevating platelet counts
6. To characterise associations of primary ITP with both arterial and venous thromboembolic events (TEs)

Data sources

1. The UK Adult ITP Registry (17 centres, 327 patients)
2. The UK Adult ITP Registry-affiliated, ^{111}In-Labelled Platelet Study Database (117 centres, 256 patients)
3. The Wellcome Trust Case-Control Consortium (WTCCC) 1958 British Birth Cohort (3,000 individuals)
4. The General Practice Research Database (GPRD) (500+ centres, 4 million+ patients)
5. The UK ITP Support Association Health-Related Lifestyle Survey (790 patients)
6. Systematic reviews of published epidemiological studies

Methods and Results

SNP Study

Caucasian patients from the UK Adult ITP Registry were gender-matched (1:3) with healthy controls from the WTCCC 1958 British Birth Cohort. Six functional candidate SNPs in cytokine or cytokine receptor genes were measured in cases and retrieved for controls from the European Genome-phenome Archive. Associations were evaluated using logistic regression models. A statistically significant per allele odds ratio (OR) of 1.34 (95% confidence interval [CI], 1.03-1.75) was observed for *TNFA* -308 g>a, implicating increased disease susceptibility among carriers of the rare allele.

^{111}In Study

The effectiveness of autologous ^{111}In-labelled platelet sequestration studies in predicting short (1-3 months), medium (6-12 months), and long-term (last follow-up) complete response (CR; count < 100 × 10^9/L) to splenectomy in patients with primary ITP was evaluated using multivariable logistic regression models. Significant adjusted (gender, age at splenectomy, and mean platelet lifespan) ORs were uncovered for short (7.47 [95% CI, 1.89-29.43], medium (4.85 [95% CI, 1.04-22.54]) and long-term (5.39 [95% CI, 1.34-21.65]) CR in patients with purely or predominantly splenic versus mixed or hepatic splenic platelet sequestration, highlighting the utility of platelet sequestration studies as an adjunct predictive tool prior to splenectomy.

H. pylori Eradication Study

A systematic literature review was conducted of studies evaluating the effects of *H. pylori* eradication on platelet count in patients with primary ITP. Twenty-five studies were identified, encompassing 696 evaluable patients. The weighted mean complete response (count > 100 × 10^9/L) and overall response (platelet count > 30 × 10^9/L) were 42.7% (95% CI, 31.8%-53.9%) and 50.3% (95% CI, 41.6%-59.0%), respectively. Observed responses were higher in countries with a higher prevalence of *H. pylori* infection and in patients with milder thrombocytopenia. These findings support the benefits of *H. pylori* detection and eradication in patients with primary ITP.

Health-Related Lifestyle Study

A 43-question, closed-field questionnaire was used to identify health-related lifestyle concerns among patient members of the UK Adult ITP Support Association. Completed surveys were returned by 790 (44.7%) members, with nearly one-third of adults reporting having an elective surgery delayed due to a low platelet count and experiencing difficulty in obtaining travel insurance. Notably, 12.5% of all patients reported "always" or "often" missing work or school due to fatigue. These results highlight several promising avenues for future health-related quality of life (HRQoL) research.

Thromboembolic Event Studies

Using the GPRD, 1,070 adults with primary ITP were matched (1:4) with 4,280 ITP-free controls by age, gender, practice, and observation time. Comparative risks of TEs were assessed using Cox proportional hazards models. Over a median time of 47.6 months (range: 3.0-192.5 months), adjusted hazard ratios of 1.58 (95% CI, 1.01-2.48) and 1.37 (95% CI, 0.94-2.00) were found for venous and arterial TEs, respectively. A similar investigation was conducted comparing age and sex-stratified incidence rates of TEs among patients in the UK Adult ITP Registry with population-based estimates for the general adult population, yielding significant standardised rate ratios of 2.43 (95% CI, 1.01-5.83) and 2.45 (95% CI, 1.48-4.06) for venous and arterial TEs, respectively. These results collectively highlight an increased risk of venous and arterial TEs in adults with primary ITP.

Conclusions

Primary ITP is a pro-inflammatory (*i.e.*, TH-1-mediated), pro-thrombotic disease in adults. Available evidence supports testing for and eradication of *H. pylori* infection in patients with primary ITP and the utility of autologous ^{111}In-labelled platelet sequestration studies in identifying patients likely to respond to splenectomy. The development of a prospective, international registry will help assemble the sample sizes needed for promising further research, namely genome-wide disease association studies and investigations into the effects of the eradication of strain-specific *H. pylori* infection on platelet count, the precise relative risk of non-response to splenectomy in patients with mixed or hepatic platelet sequestration, and the associations of TEs with primary ITP in antiphospholipid antibody-negative.

This dissertation is dedicated to my family:

Aai, Baba, Sarika, Ravi, and Hobbes

CONTENTS

Preface

The aim of this thesis was to further current understanding of primary immune thrombocytopenia (ITP) pathogenesis, treatment effectiveness, and co-morbid burden among adults. Results from investigations evaluating health-related lifestyle concerns among patients, the utility of a prognostic radioisotope test prior to splenectomy, the effectiveness of *Helicobacter pylori* (*H. pylori*) eradication in platelet count elevation, and primary ITP associations with both single nucleotide polymorphisms (SNPs) and thromboembolic events (TEs) are presented in 9 chapters. This thesis is the result of my own work and includes nothing done in collaboration except where specifically indicated (see Acknowledgements).

The majority of data used in the aforementioned investigations was derived from the United Kingdom (UK) Adult ITP Registry, a repository of clinical data and biological samples of adult patients with primary ITP, which I developed and launched under the supervision of Dr. Drew Provan as part of my doctoral studies. Principle funding for the Registry was provided by GlaxoSmithKline (GSK). The pharmaceutical company had recently developed a thrombopoietin-agonist (eltrombopag) for the treatment of primary ITP and was interested in securing additional epidemiological data on the disease in the adult population. As will be discussed at length in Chapter 9 of this thesis, industry sponsorship of research is a necessary, albeit inherently problematic, reality of academic medicine. Cognizant of the potential influence of GSK on the research presented herein, I affirm that the Registry-specific analyses were designed independently by me and that the conclusions reached from the data generated were mine alone.

6 September 2010

Signature Date

Acknowledgements

Upon the completion of my M.Phil. studies in August 2006, I approached Drs. Jamie Robinson (GSK) and Julian Higgins (University of Cambridge) regarding my interest in pursuing doctoral work in epidemiology. Fortuitously, they placed me in contact with Drs. Drew Provan (The Royal London Hospital) and Simon Sanderson (University of Cambridge), who agreed to serve as co-supervisors for my proposed Ph.D. on disease pathogenesis, treatment effectiveness, and co-morbid burden among adults with primary immune thrombocytopenia (ITP). Drs. Provan and Sanderson have provided invaluable support and guidance throughout my studies. However, in January 2010, Dr. Sanderson was sadly forced to take early retirement on poor health grounds. Dr. Provan has nonetheless provided critical feedback on the chapters presented herein. I am in particular thankful for his mentorship, without which this work would not have been possible.

I would also like to express my appreciation to Dr. Higgins for serving as an acting co-supervisor in Dr. Sanderson's absence, to Professor Adrian Newland for his timely advice, and to Dr. Sebhat Erqou for his statistical guidance. A number of individuals have contributed to the investigations comprising this thesis. Their roles are gratefully acknowledged below.

Chapter 1

I composed the text, produced the tables, and adapted the figures in this chapter. Dr. Erqou commented helpfully.

Chapter 2

I composed the text and produced the tables and figures in this chapter. I specified the parameters of the Registry, secured ethical approval and funding for its initiation, directed centre and patient recruitment, and led the extraction of data from hospital medical records at Barts and The London NHS Trust. I further devised the analysis plan of baseline data, which was performed in Stata by Dr. Erqou. Dr. Yu-mei Chang and part-time student research assistants helped with the extractions. A full list of past and present student research assistants may be found at the following site:
http://www.ukitpregistry.com/UKITPContactpage/page20/page20.html.

Chapter 3

I composed the text and produced the tables and figures in this chapter. Dr. Momin Ahmed genotyped the case samples, and I drafted the application to secure data on controls from the Wellcome Trust Case Control Consortium. I sex-matched (1:3) cases with controls, coded Registry data in accordance with international consensus guidelines, and performed the analyses. Tie Sing Fong, Helen Ngu, and Sarene Saw assisted me with coding, and Dr. Erqou provided statistical guidance. Professor John Semple; Drs. Paul Pharoah, Dimitri Bennett, and Jim Bussel; and Ravi Sarpatwari commented helpfully.

Chapter 4

I composed the text and produced the tables and figures in this chapter, the contents of which have recently been accepted for publication by the *British Journal of Haematology*. With assistance from Dr. Ravin Sobnack and Nish Fernando, I secured scan data from the Department of Nuclear Medicine at St. Bartholomew's Hospital. I additionally steered participant follow-up, receiving help from Dr. Provan, Professor Newland, and David Tai. I devised and conducted the analyses. Professor Newland and Dr. Bennett commented helpfully.

Chapter 5

In April 2007, I formulated the idea of conducting a systematic review of the effects of eradication therapy on platelet counts in *H. pylori*-infected patients with primary ITP and worked with Dr. Jae Park, a visiting research fellow from Massachusetts General Hospital, to perform a literature search and subsequent data extraction. Dr. Roberto Stasi had, meanwhile undertaken a similar course of action with Dr. Maria Laura Evangelista. Following consultation with Dr. Provan, I happily merged my efforts with those of Dr. Stasi. I devised and performed the primary meta-analyses, serving as second author on the final report published by *Blood* in May 2009. Portions of the text, tables, and figures presented in this chapter stem from this report. I further led the authors' response to a critical review of the report from Dr. Mark Crowther and colleagues.

Chapter 6

I composed the text, produced the tables, and adapted the figures in this chapter. Shirley Watson, Howard Anderson, and Dr. John Grainger conceived the health-related lifestyle study, constructed the survey instrument, and extracted data from returned questionnaires. I performed the study analyses and authored the study report, which has recently been e-published by the *British Journal of Haematology*. Drs. Erqou and Higgins commented helpfully.

Chapter 7

I composed the text and produced the tables and figures in this chapter. I designed the study in collaboration with Dr. Bennett and Amit Shukla and formulated the analysis plan, carried out in SAS by Dr. John Logie and Amit Shukla. I interrogated the data, devised further post-hoc analyses, and authored the report of study findings, which were published in *Haematologica* in July 2010. Drs. Provan, Bennett, and Logie and Professor Newland commented helpfully.

Chapter 8

I composed the text and produced the tables and figures in this chapter. I designed the study and further developed the statistical methods and performed the analyses in collaboration with Dr. Erqou. Drs. Mike Colopy and Sarika Gupta commented helpfully.

Chapter 9

I composed the text and produced the tables and figures in this chapter. I authored a proposal for a decentralised international adult ITP registry, which will be piloted this fall with the development of an autonomous, affiliated Pan-American Registry. I am further working to develop, test, and validate a prognostic model for disease trajectory in primary ITP.

List of Abbreviations (I of II)

ASH: American Society of Hematology

AIHA: Autoimmune haemolytic anaemia

BCSH: British Committee for Standards in Haematology

BSH: British Society for Haematology

CagA: Cytotoxic-associated gene A

CI: Confidence interval

CNV: Copy number variation

CR: Complete response

CRF: Chart review form

CT: Computed tomography

DVT: Deep vein thrombosis

EGA: European Genome-phenome Archive

EMA: European Medicines Agency

FBC: Full blood count

FDA: Food and Drug Administration

GP: Glycoprotein

GPRD: General Practice Research Database

GSK: GlaxoSmithKline

HCV: Hepatitis-C virus

HIV: Human immunodeficiency virus

HLA: Human leukocyte antigen

HR: Hazard ratio

HRQoL: Health-related quality of life

HWE: Hardy-Weinberg equilibrium

ICD: International Classification of Diseases

ICMS: Institute of Cell and Molecular Science

IFN: Interferon

Ig: Immunoglobulin

IL: Interleukin

IR: Incidence rate

IRR: Incidence rate ratio

IS: Ischaemic stroke

ITP: Immune thrombocytopenia

ITP-PAQ: ITP-Patient Assessment Questionnaire

IVIg: Intravenous immunoglobulin

KIT: Kids' ITP Tool

MDS: Myelodysplastic syndromes

MDSAS: Medical Data Solutions and Services

MeSH: Medical Subject Heading

MI: Myocardial infarction

MPLS: Mean platelet lifespan

NHS: National Health Service

NIHR: National Institute for Health Research

OR: Odds ratio

List of Abbreviations (II of II)

OXMIS: Oxford Medical Information System

PE: Pulmonary embolism

PPV: Positive predictive value

PVT: Portal vein thrombosis

RCT: Randomised-controlled trial

REC: Research ethics committee

RES: Reticuloendothelial system

SF: Short form

SLE: Systemic lupus erythematosus

SNP: Single nucleotide polymorphism

SRR: Standardised rate ratio

TE: Thromboembolic event

Th: T-helper

TIA: Transient ischaemic attack

TNF: Tumour necrosis factor

TPO: Thrombopoietin

TTP: Thrombotic thrombocytopenic purpura

UA: Unstable angina

UBT: Urea breath test

UK: United Kingdom

UKCRN: United Kingdom Clinical Research Network

US: United States

WTCCC: Wellcome Trust Case Control Consortium

Chapter 1: Introduction

Summary

Primary immune thrombocytopenia (ITP) is a relatively rare autoimmune disease characterised by autoantibody-mediated platelet destruction, T-cell-mediated platelet lysis, and suboptimal platelet production in the absence of a demonstrable cause, leading to decreased peripheral blood platelet counts (< 150 × 10^9/L) or thrombocytopenia. The disease typically runs a chronic (> 6 months) course in adults and exhibits considerable phenotypic heterogeneity. Thus, although one-fourth of patients are asymptomatic at diagnosis (*i.e.*, diagnosed incidentally), clinically relevant morbidity, including major haemorrhage, is not uncommon. Despite a surge in research over the past decade, considerable epidemiological questions remain concerning primary ITP pathogenesis, treatment effectiveness, and co-morbid burden in adults. This thesis aimed to advance current understanding in these areas by characterising associations of primary ITP with both candidate single nucleotide polymorphisms (SNPs) and thromboembolic events, by assessing the utility of autologous ^{111}In-labelled platelet sequestration studies prior to splenectomy, and by gauging the effectiveness of eradication therapy in elevating platelet counts in *H. pylori*-infected patients.

Background

Primary immune thrombocytopenia (ITP) is an autoimmune disease characterised by autoantibody-mediated platelet destruction, T-cell-mediated platelet lysis, and suboptimal platelet production in the absence of a demonstrable cause.[1,2] These processes result in a decreased peripheral blood platelet count (*i.e.*, thrombocytopenia), defined as less than 150×10^9/L.[3-5*] The course of primary ITP in children is typically acute (< 6 months[3-5*]),[6] whereas among adults it is predominantly chronic.[7] The clinical manifestations of the disease are heterogeneous. While approximately one-fourth of adult patients are asymptomatic at diagnosis (*i.e.*, incidentally diagnosed), petechiae, purpura, and mucosal bleeds are not uncommon.[8,9] The past decade has witnessed a marked increase in research into the disease, fuelled in part by the successful development and testing of novel therapeutic agents. However, despite these efforts, considerable gaps in our understanding of primary ITP pathogenesis, treatment effectiveness, and co-morbid disease burden remain.[2]

Clinical Definition

Primary ITP is clinically defined by the presence of thrombocytopenia in the absence of demonstrable pathogenic, therapeutic, systemic (autoimmune diseases or malignancies), or congenital causes. As such, primary ITP can only be diagnosed through thorough clinical evaluation, which both past[3,4] and present guidelines[2] recommend be based upon a detailed history, physical examination, full blood count (FBC), and blood film examination. These investigations may direct clinicians toward alternate diagnoses; a comprehensive history, for example, may identify a dietary cause of thrombocytopenia[10] while a blood film may reveal a *MHY9*-related disorder (Figure 1-1).

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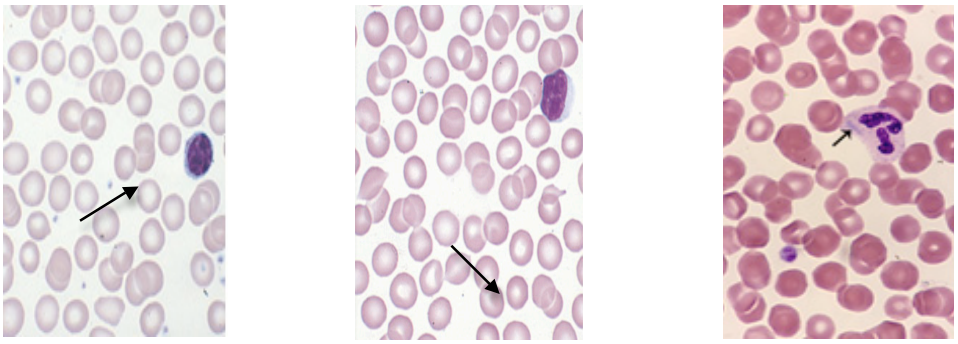
[*] A panel of internationally recognised ITP specialists have recently recommended that the definitions of thrombocytopenia and chronic disease be changed to less than 100×10^9/L and greater than one year, respectively. They have further recommended that the formal name of the disease be changed from idiopathic thrombocytopenic purpura to primary immune thrombocytopenia to highlight its autoimmune aetiology and heterogeneous clinical manifestations. See *Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113:2386-2393.*

Owing to potentially significant differences in management, particular care must be given to distinguishing primary ITP from hepatitis-C (HCV) and human immunodeficiency virus (HIV)-induced thrombocytopenias. Screening for these two conditions is therefore recommended for all patients during initial work-up.[2] Bone marrow aspiration and trephine biopsy are further advised for elderly (> 60 years old at presentation) patients due to their elevated risk of myelodysplastic syndromes (MDS) and haematological malignancies.[2-4] Finally, as systemic autoimmune diseases (*e.g.*, systemic lupus erythematosus [SLE]) may initially manifest as isolated thrombocytopenias, periodic revaluation of the diagnosis of primary ITP is necessary.[11]



(a) **(b)** **(c)**

Figure 1-1: Blood films of a healthy donor (a) and patients with primary ITP (b) and May-Hegglin Anomaly (c). A reduction in platelets (view arrows) is evident in a blood film of a patient with primary ITP in comparison with that of a healthy donor. May-Hegglin anomaly, a congenital thrombocytopenic disease, may be distinguished from primary ITP by the presence of giant platelets on a blood film. *Films (a) and (c) adapted from Lichtman's Atlas of Hematology,[14] film (b) courtesy of Dr. Drew Provan.*

Patients in whom the onset of thrombocytopenia is coupled with the concurrent or sequential development autoimmune haemolytic anaemia (AIHA) comprise a disease subclass known as Evans syndrome.[†13] Higher rates of morbidity and mortality have been observed in adult patients with Evans syndrome compared to those with isolated thrombocytopenia.[14]

[†] Some ITP specialists class AIHA and *Helicobacter pylori* (*H. pylori*) infection-associated ITP as secondary ITP. *See Cines DB, Bussel JB, Liebman HA, Luning Prak ET. The ITP syndrome: pathogenic and clinical diversity. Blood. 2009;113:6511-6521.* However, as conclusive evidence has not yet emerged linking the thrombocytopenia to the pathogenesis of these conditions, they are considered specialised cases of primary ITP for the purposes of this thesis.

Pathogenesis

Traditional Model: Autoantibody-Mediated Destruction by the Reticuloendothelial System

Almost 60 years have passed since Harrington *et al.*'s[15] classic experiment in which blood samples from patients with primary ITP were injected into compatible, healthy volunteers, resulting in an immediate reduction in platelet count (Figure 1-2). These findings prompted Harrington to hypothesise the existence of an anti-platelet factor in the globulin fraction of plasma of patients with primary ITP. Through the work of McMillan *et al.*,[16] Dixon *et al.*,[17] and van Leeuwen *et al.*,[18] this factor was successfully isolated and identified as anti-platelet autoantibody. Studies have subsequently shown that adult patients with primary ITP most commonly exhibit autoantibodies to platelet glycoprotein (GP)IIb/IIIa and GPIb/IX, though those to GPIa/IIa, GPIV, and GPV have also been observed.[1,19] The chief class of these antibodies is immunoglobulin (Ig)G, namely the complement-fixing IgG1 and IgG3 isotypes.[20,22]

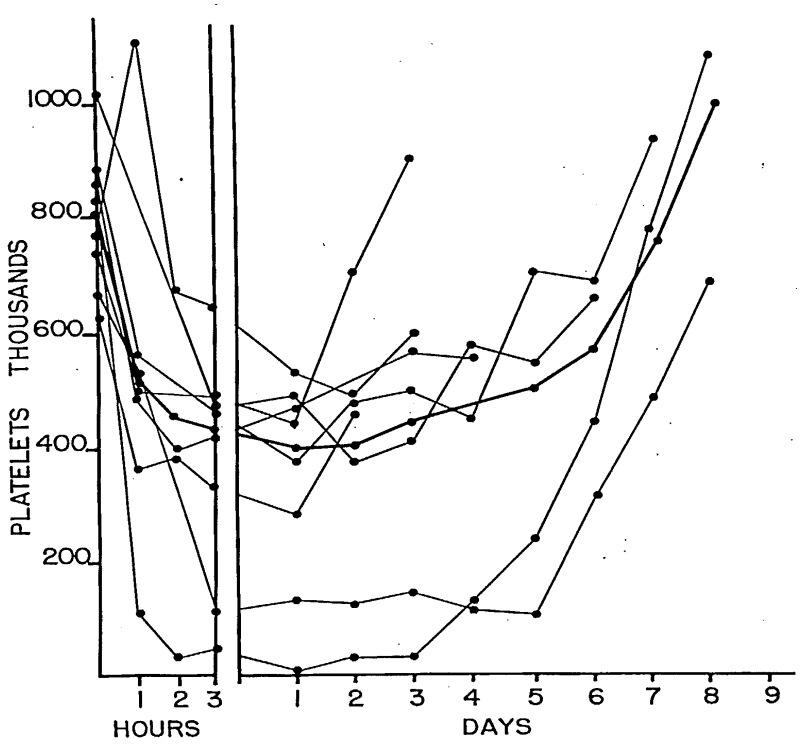


Figure 1-2: Results from Harrington *et al.*'s classic 1951 experiment. Blood from patients with primary ITP was injected into healthy volunteers[‡], resulting in a marked reduction in platelet count within hours. *Adapted from Harrington WJ, Minnich V, Hollingsworth JW, Moore CV. Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. J Lab Clin Med. 1951;38:1-10.*

[‡] Patients with inoperable neoplasms also served as controls in the study.

The discovery of anti-platelet autoantibodies was paralleled by observations of shortened mean platelet lifespan (MPLS) in patients with primary ITP relative to healthy individuals. Harker,[22] for example, used allogeneic, ^{51}chromium (^{51}Cr)-labelled platelets to document a MPLS of 0.3 ± 0.4 days in 16 patients with primary ITP versus 9.9 ± 0.6 days in 15 healthy controls. The combination of these findings led investigators to propose autoantibody-mediated, peripheral platelet destruction by the reticuloendothelial system (RES) as the underlying mechanism of disease in primary ITP, the traditional model of primary ITP pathogenesis.

Revised Model: Increased Destruction & Suboptimal Production & Defective Regulation

The adequacy of the traditional model, however, was challenged when allogeneic platelets were substituted with autologous platelets in radioisotopic studies. Using autologous ^{111}Indium (^{111}In)-labelled platelets, Ballem *et al.*[23] reported that platelet turnover, a proxy for platelet production, was decreased[§] in 7 of 17 (41.2%) untreated patients with primary ITP in comparison with 15 healthy controls. A higher MPLS was further noted in patients with primary ITP than in previous investigations. To explore this discrepancy, Ballem *et al.* examined the results of 4 patients for whom both allogeneic and autologous platelet radioisotopic studies had been performed. A significant difference in MPLS (autologous: 3.6 ± 1.4 days vs. allogeneic: 1.2 ± 1.2 days; $p = 0.017$) was observed, leading to speculation that the results of earlier investigations may have been biased by alloimmunisation.[23,24] Subsequent findings of impaired *in vitro* megakaryocytopoiesis following the addition of plasma from adults and children with primary ITP have further underscored the role of suboptimal platelet production in primary ITP pathogenesis.[25,26]

Recent studies have uncovered an even greater diversity of mechanisms in primary ITP. Noting that prior investigations were unable to detect anti-platelet autoantibodies in 30% to 50% of diagnosed patients,[27,28] Olsson *et al.*[29] questioned whether T-cell-mediated cytotoxicity constituted an alternate pathogenic pathway. They found evidence in support of this theory in the form of increased expression of genes associated with cell-mediated immunity and elevated rates of *in vitro* platelet destruction by $CD14^{-}CD19^{-}$ mononuclear cells (T cells and natural killer cells) in

[§] More than 2 standard deviations lower than the mean platelet turnover seen in healthy controls.

patients with active primary ITP compared with both patients with remitted primary ITP and healthy controls.[29]

This report was followed by observations implicating defective T regulatory cells (Tregs) in the loss of self-tolerance and, thus, disease onset in patients with primary ITP. While Yu *et al.*[30] found no difference in the number of circulating Tregs in 17 patients with chronic primary ITP and 16 age-matched, healthy controls, they noted a statistically significant ($p < 0.05$), twofold decrease of Treg immunosuppressive activity *in vitro*.[30] A revised mechanistic model of primary ITP must then encompass not only autoantibody-mediated platelet destruction and suboptimal platelet production, but also T-cell-mediated platelet lysis and impaired self-tolerance, mechanisms that may or may not occur in tandem within an individual patient.

Cytokine Profiles in Primary ITP: A Th-1 Disease

Cytokines are powerful messenger proteins of low molecular weight and serve as critical effectors of the aforementioned mechanisms. Under the Mosmann-Coffman[31] model of T-helper (Th) cells, immunological homeostasis is maintained through the balance of cytokines secreted by Th1 cells, which promote pro-inflammatory, cell-mediated and complement-fixing IgG isotype responses, and Th2 cells, which elicit immediate-type hypersensitivity, augmenting humoral defense. [21,32,33] Past investigations have demonstrated Th1 polarisation in primary ITP. Wang *et al.*[34] reported a significantly ($p = 0.002$) higher Th1/Th2 ratio among 20 adults with active chronic primary ITP than in 20 healthy adult volunteers. Panistas *et al.*[35] further demonstrated an inverse correlation ($r^2 = 0.251$, $p = 0.017$) of this ratio with platelet count when evaluating 11 healthy adult controls and 21 adult patients with chronic primary ITP. Finally, in an investigation of 34 children with chronic or acute primary ITP, Semple *et al.*[36] were unable to detect any levels of interleukin (IL)-4 or IL-6, prototypical Th2 cytokines. While these data highlight primary ITP as a pro-inflammatory TH1 disease, the potential genetic contribution to this imbalance remains unclear.

Elements of a Perfect Storm: Genetic Susceptibility and an Environmental Trigger

It is generally accepted among immunologists that while genetic susceptibility may be necessary, it is not sufficient for the development of autoimmune disease within an individual. Ermann and Fathman,[37] for instance, note that concordance for a autoimmune disease is less than 50% among monozygotic twins. It has therefore been widely postulated that an environmental trigger (*e.g.*, an infection) must also be present to initiate the autoimmune pathway via such mechanisms as molecular mimicry or Treg inhibition.[38]

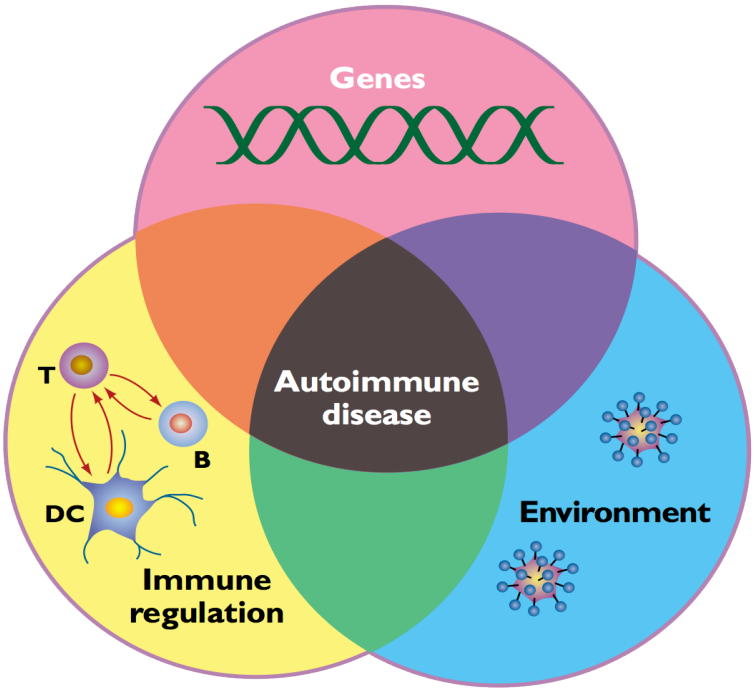


Figure 1-3: The aetiology of autoimmune disease is multi-factorial. It is hypothesised that the onset of autoimmune disease requires both a genetic predisposition to immune dysregulation and an environmental trigger to initiate pathogenesis. *Adapted from Ermann J, Fathman CG. Autoimmune diseases: genes, bugs and failed regulation. Nat Immunol. 2001;2:759-761.*

One such hypothesised trigger of primary ITP is *Helicobacter pylori* (*H. pylori*) infection. In a 1998 letter in *The Lancet*, Gasbarrini *et al.*[39] reported that 11 of 18 patients with ITP evaluated at their institution tested positive for *H. pylori* infection. At 2 and 4 months following the administration of triple-therapy,[**] a statistically significant increase in platelet counts from baseline was observed only in patients in

[**] Triple-therapy is the standard treatment for *H. pylori* infection and consists of a proton pump inhibitor and two classes of antibiotics.

whom *H. pylori* was eradicated (N = 8).[39] Furthermore, 6 (75.0%) of these patients were no longer found have detectable anti-platelet autoantibodies.[39] A host of single-centre trials have been conducted following the publication of this study;[40-43] further assessment of their aggregate results is warranted.

Descriptive Epidemiology

The descriptive epidemiology of primary ITP in adults remains poorly investigated. A systematic review of autoimmune diseases conducted by Jacobson *et al.*[44] in 1997 found no population-based studies of primary ITP in the medical literature. Such studies either directly evaluate or are representative of all individuals within a defined geographic region and are therefore critical for accurate measures of disease frequency.

A total of 6 population-based studies, 3 in the United Kingdom (UK),[45-47] 2 in the United States (US),[48,49] and 1 in Denmark,[8] have since been conducted and published in peer-reviewed journals (Table 1-1). The number of new cases, or incidence,[††] of primary ITP was estimated by 4[8,45-47] of these investigations. As shown in Table 1-2, Schoonen *et al.*[46] and Abrahamson *et al.*[47] utilised comparable classification codes to identify adult patients with primary ITP in the General Practice Research Database (GPRD), a repository of clinical data on over 4 million patients across 500 Primary Care centres in the UK. They reported an incidence rate of between 3.8[47] and 3.9[46] per 100,000 adult person-years. To capture the frequency of the disease most likely to require active clinical management, Frederiksen *et al.*[8] and Neylon *et al.*[45] restricted their disease populations to patients with platelet counts below 50×10^9/L, documenting an annual incidence of 2.64 and 1.6 per 100,000 adults, respectively. All four studies noted a sharp rise in the incidence with increasing age and a moderate female preponderance (female-to-male ratio range: [1.2-1.7]:1) consistent with other autoimmune diseases.[50] By contrast, population-based studies have captured incidence rates of 0.15 per 100,000 person-years[51] for acquired haemophilia and 0.45 per 100,000 person-years[52] for idiopathic thrombotic thrombocytopenic purpura (TTP). These estimates collectively suggest that primary ITP, though rare, is nevertheless among the more commonly experienced autoimmune haematological diseases in adults.

[††] Use of the term incidence rate is conventionally limited to measures of frequency in which the denominator is at risk person-time (*e.g.*, person-years).

The lower annual incidence observed in Neylon *et al.*'s study is likely a result of their requirement of a bone marrow aspirate and trephine biopsy in all patients for diagnostic confirmation. However, the possibility of ethnic and geographic differences cannot be excluded. A paucity of data exists on the subject. In a 2005 review, Terrell *et al.*[53] were only able to secure 6 US studies with 10 or more patients with primary ITP identified by race. The data were suggestive of a possible lower prevalence, or number of new and existing cases,[‡‡] of primary ITP among African-Americans. This hypothesis was further explored by Landgren *et al.*,[54] who failed to detect a disparity between white and African-American male veterans, an admittedly non-population-based cohort. To date, no systematic comparative investigation has been published on international differences in the incidence or prevalence of primary ITP in adults.

Estimation of the prevalence of chronic primary ITP among adults has proven particularly challenging owing to uncertain accuracy of ascertainment methods within non-disease-specific databases. Segal and Powe[48] and Feudjo-Tepie *et al.*[49] both used the International Classification of Diseases (ICD)-9 287.3 code in health insurance claims in the US to derive estimates of this parameter. The diagnostic accuracy of the code in such a setting had been subject to a prior validation study, which documented a sensitivity and specificity[§§] of 84% and 66% in outpatients and 100% and 89% in inpatients respectively when compared with hospital medical records.[55]

To exclude patients with acute disease, Segal and Powe and Feudjo-Tepie *et al.* required entry of 2 distinct ICD-9 287.3 codes separated by great than 30 and 180 days, respectively.[48,49] As Segal and Powe did not provide an overall prevalence of primary ITP among adults, their age-specific estimates were pooled using a DerSimonian and Laird random-effects meta-analysis, yielding an overall prevalence of 10.0 per 100,000 adults (Table I-I) over one year. Although this estimate appears considerably different from that reported by Fuedjo-Tepie *et al.*, 24.1 per 100,00 adults,[49] the latter prevalence encompassed 5-year period and included patients over 65 years of age. Segal and Powe's estimate would prove more comparable were one

[‡‡] Prevalence can be measured at a given time (point) or over a specified interval (period).
[§§] The terms sensitivity and specificity refer to the probability of correct classification of patients with and without disease respectively, in this instance patients with and without primary ITP.

to account for incident cases of primary ITP in adults over an additional 4-year period:[***] 20.6 per 100,000 adults.

Table 1-2: Population-Based Studies on Primary ITP in Adults

Authors (Publication Year)	Country	Data Source	Criteria	Frequency Estimate
Incidence Rate of Primary ITP (95% Confidence Interval) Per 100,000 Person-Years: Unadjusted				
Schoonen *et al.*[46] (2008)	UK	General Practice Research Database	•Age: > 18 years •Read codes: 42P2.11, 42P..00, 42PZ.00, D313.11, D313.12, D313000, D313012; OXMIS[†††] codes: 2871C, L_146N •Period: First code between 1990-2005	**3.8** (3.6-4.1)
Abrahamson *et al.*[47] (2009)	UK	General Practice Research Database	•Age: ≥ 18 years •Read codes: 42P2.11, D313.11, D313.12, D313000, D313012; OXMIS codes: 2871C •Period: First code between 1992-2005	**3.9** (3.6-4.1)
Annual Incidence of Primary ITP (95% Confidence Interval) Per 100,000 Persons Per Year: Unadjusted				
Frederiksen *et al.*[8] (1999)	Denmark	Retrospective Cohort County of Funen	•Age: ≥ 15 years; count[‡‡‡] < 50 × 10^9/L •American Society of Hematology[3] criteria •Period: April 1973 – December 1995	**2.64** (2.29-2.98)
Neylon *et al.*[45] (2003)	UK	Prospective Cohort Northern Health Region	•Age: > 16 years; count < 50 × 10^9/L •American Society of Hematology[3] criteria, bone marrow aspirate & trephine biopsy •Period: January 1993 – December 1999	**1.6** (N/A)[§§§]
Period Prevalence of Chronic, Primary ITP (95% Confidence Interval) Per 100,000 Persons: Unadjusted				
Segal *et al.*[48] (2006)	USA	Medical Care Database Maryland Health Care Commission	•Age: 18 < X < 65 years •ICD-9* code: 287.3; ≥ 2 codes separated by > 30 days •Period: 2002	**10.0**[****] (5.4-14.7)
Fuedjo-Tepie *et al.*[49] (2008)	USA	Integrated Healthcare Information System Database	•Age > 18 years •ICD-9 code: 287.3; ≥ 2 codes separated by > 6 months •Period: January 2002 – December 2006; continuous enrolment in 2004 required; first code ≤ 2004, second code ≥ 2004	**24.1** (23.4-24.8)

[***] Assuming a conservative annual incidence of 2.64 per 100,000 adults (Frederiksen *et al.*), 10.6 incident cases of primary ITP would be expected over a four-year period.

[†††] OXMIS: Oxford Medical Information System; ICD: International Classification of Diseases

[‡‡‡] Frederiksen *et al.* additionally reported incidence rates using a threshold of less than 100 × 10^9/L.

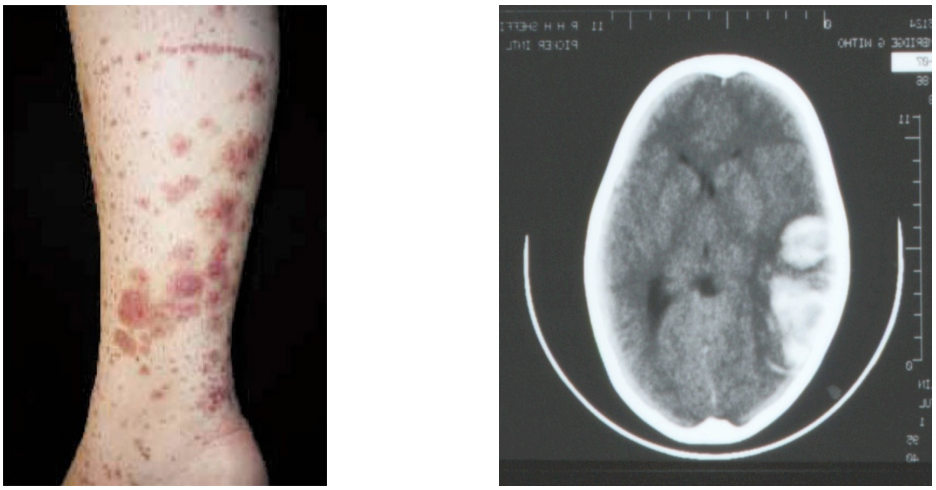
[§§§] Neylon *et al.* did not provide a 95% confidence interval.

[****] Segal *et al.* did not provide an overall prevalence of primary ITP among adults in their study but instead reported the following age-specific estimates: 19-24 years: 4.1 (2.0-7.3), 25-34 years: 9.3 (5.8-14.0), 35-54 years: 11.0 (9.1-13.0), 55-64 years: 16.0 (13.0-20.0) per 100,000 adults over one year. These estimates were pooled using a Der-Simonian and Laird random-effects meta-analysis.

Bleeding Manifestations

The principal bleeding manifestations of primary ITP are petechiae and purpura, or pinpoint haemorrhages and ecchymoses, which result from vessel rupture or leakage (Figure 1-4[a]). Mucosal haemorrhages such as epistaxis, gum bleeds, and menorrhagia are also commonly observed, albeit with decreased frequency. The incidence of major bleeding events (*e.g.*, intracranial haemorrhage [Figure 1-4(b)]) among adults with primary ITP is perceived to be low, with a long-term observational study estimating a 1.6%[45] cumulative incidence of fatal haemorrhage over a median interval of 5 years (range: 0.5-6.5 years).

The platelet count remains the best-known predictor of bleeding events in primary ITP. Portielje *et al.*[9] reported no such events in patients with moderate thrombocytopenia (> 30 x 10^9/L) when evaluating 152 consecutive adults with primary ITP over a median time of 9.4 years (range: 0.2-22.6 years). Similarly, in a retrospective study of 208 patients spanning a median period of 7.7 years (4.0-12.6 years), Stasi *et al.*[7] observed bleeding events in only 5 (5.7%) patients with a presenting count greater than 50 × 10^9/L.



(a) **(b)**

Figure 1-4: Images of cutaneous bleeding (a) and an intracranial haemorrhage from a computerised tomography (CT) scan (b). Petechiae and purpura are commonly observed in adult patients with primary ITP. The risk of intracranial haemorrhage, however, is low. *Images courtesy of Dr. Drew Provan*

Although an increased risk of major bleeding events among patients with severe thrombocytopenia (< 30 x 10^9/L) has been well documented,[7-9,56] both its magnitude and distribution remain controversial. In a meta-analysis of cohort studies on primary ITP, Cohen *et al.*[56] estimated an incidence rate of 0.0162 to 0.0389 fatal haemorrhages per severely thrombocytopenic patient-year, which translates to a seemingly high 10-year cumulative risk of 16.2% to 38.9%. Porteilje *et al.*[9] reported a comparable incidence rate of fatalities, 0.019 per patient-year, but noted that bleeding events and infection contributed equally to cause of death within their cohort

The risk of major haemorrhage in this population, moreover, appears heterogeneous. Frederiksen *et al.*, for example, reported no episodes of severe[††††] bleeding in 16 patients with platelet counts persistently below 50 x 10^9/L over a median of 24 months.[8] Additionally, numerous anecdotal reports of asymptomatic patients with platelet counts less than 20 x 10^9/L abound. A highly sensitive instrument capable of identifying individuals in whom such events are likely to occur would therefore prove of considerable benefit in the management of adult patients with primary ITP.

Disease Management

First-Line Treatments: Corticosteroids, Intravenous Immunoglobulin, and Anti-D

Prednisolone[‡‡‡‡] remains the most common first-line treatment for patients with primary ITP.[1,57] In a retrospective study of 208 adults with primary ITP over a median time of 7.7 years (range: 4.0-12.6 years), Stasi *et al.*[7] observed a complete remission (platelet count > 120 × 10^9/L, one month following the discontinuation of therapy) in 47 (38.8%) patients initially receiving this treatment. However, responses were maintained in only 11 (8.6%) patients at last follow-up.[7] Multiple observational studies have documented a comparable decline in long-term success among initial responders to prednisolone.[3] Dexamethasone and methylprednisolone comprise additional members of the corticosteroid family that have been recommended as possible first-line treatments for the management of primary ITP by an international panel of clinical specialists.[2] Although these therapeutic agents have been shown to

[††††] Frederiksen *et al.* did not define severe bleeding.

[‡‡‡‡] Prednisone is activated to prednisolone in the liver. For the purposes of this thesis, the term prednisolone shall encompass both prednisolone and prednisone.

elicit an initial response[§§§§] in 80.0% to 89.2% of adult patients in clinical trials, a substantial drop off in long-term response has been observed with them as well[58-61] Intriguingly, no randomised-controlled trials (RCTs) have been conducted to evaluate the effectiveness of corticosteroids with respect to non-treatment in adults with primary ITP.

The side effects of long-term administration of corticosteroids have been well documented in the medical literature and include weight gain, cataracts, hypertension, diabetes, and osteoporosis (among others).[2] These risks are generally well known to patients with primary ITP, who often report that the treatment for the disease is worse than the disease itself.[62] Therefore, it has now been recommended that corticosteroids be slowly tapered and stopped in adult patients who have not shown a response within 4 weeks of the initiation of treatment.[2]

The remaining first line treatments for primary ITP include intravenous immunoglobulin (IVIg) and, in Rh(D)-positive patients, anti-D, which have been hypothesised to work in part via Fc-receptor blockade.[21] Imbach *et al.*[63] first demonstrated the efficacy of IVIg as a primary ITP treatment in a RCT of 108 previously untreated children with acute disease; comparable responses (platelet counts > 100 × 10^9/L) were noted in patients receiving IVIg (39 [83.0%]) and prednisolone (36 [76.6%]). Meanwhile, in a more recent study of 28 Rh(D)-positive, non-splenectomised adults with primary ITP, Cooper *et al.*[64] reported that 12 (42.8%) patients were able to maintain platelet counts above 30 × 10^9/L without treatment for at least 6 months following anti-D therapy. Both agents are fast acting, with an estimated time to peak response of 1-3 days, and may therefore be effective in emergency situations.[2]

Second-Line Treatments: Splenectomy, Rituximab, Thrombopoietin Agonists, and More

Splenectomy has historically been the most common second-line treatment for adult patients with primary ITP who are either unresponsive to or require unacceptably high doses of corticosteroids.[57,65] In a systematic review of 135 case-series, Kojouri *et al.*[65] observed a complete response[*****] at last follow-up in two-thirds of adult patients with primary ITP. Indeed, surgery is considered by some ITP

[§§§§] An initial response was defined as a platelet count above 50 × 10^9/L by Alpdogan *et al.* and Cheng *et al.* and above by 100 × 10^9/L by Mazzuconi *et al.* and Von Dem Borne *et al.*

[*****] A complete response was defined as a platelet count above 150 × 10^9/L, or as defined in the study and at least 100 × 10^9/L, and no ITP-specific treatment 30 days post-splenectomy.

clinical specialists the only curative treatment for the disease.[66] However, the procedure is not without risk, including intrabdominal haemorrhage,[67] thromboembolic events,[68] and opportunistic post-splenectomy infections.[69] Knowledge of these risks, together with a recognised non-response to treatment among one-third of adult patients with primary ITP and limited long-term data on long-term relapse,[†††††] demonstrate the potential utility of identifying a pre-surgical predictor of success. Although a number of variables have been proposed, they have been largely met with scepticism in the absence of sufficient observational data.[65]

Table 1-3: First and Second-Line Treatments for Primary ITP[2]

Treatment	Recommended Dosing
First-Line Treatments *(In Alphabetical Order)*	
Anti-D[‡‡‡‡‡]	50-75 μg/kg
Dexamethasone	40 mg/day × 4 days/2-3 weeks × 1-4 cycles
Intravenous Immunoglobulin (IVIg)	0.4 g/kg/day × 5 days *or* 1 g/kg/day × 1-2 days
Methylprednisolone	30 mg/kg/day × 7 days
Prednisolone	0.5-2 mg/kg/day × 2-4 weeks
Second-Line Treatments *(In Alphabetical Order)*	
Azathioprine	1-2 mg/kg/day
Cyclophosphamide	Orally: 1-2 mg/kg × 7 weeks *or* Intravenously: 0.3-1 g/m^2/day × 1-3 days/2-4 weeks
Cyclosporine	5 mg/kg/day × 6 days *then* 2.5-3 mg/kg/day
Danazol	200 mg *b.d.*[§§§§§]
Dapsone	75-100 mg/day
Mycophenolate	100 mg *b.d.*
Rituximab	375 mg/m^2/week × 4 weeks
Splenectomy	N/A
TPO[******] receptor agonist: eltrombopag	25-75 mg/day
TPO receptor agonist: romiplostim	1-10 μg/kg/week
Vinca alkaloid: vinblastine	10 mg/week × 3 weeks
Vinca alkaloid: vincristine	1-2 mg/week × 3-6 weeks

[†††††] The median follow-up time in Kojouri *et al.'s* systematic review was 2.8 years (0.3-12.8 years).
[‡‡‡‡‡] Intravenously administered anti-D
[§§§§§] Twice daily
[******] Thrombopoietin

Recent additions to the second-line treatment arsenal include rituximab and the recently approved thrombopoietin (TPO) receptor agonists, romiplostim and eltrombopag. The latter treatments stimulate platelet production by megakaryocytes while rituximab, a chimeric monoclonal anti-CD20 antibody, has been shown to improve Treg numbers and function through B cell depletion.[70-72]

A host of immunosuppressive, antibacterial, and anti-mitotic agents comprise the remaining second-line treatments for patients with primary ITP. A full listing of first and second-line treatments and their recommended dosing are shown in Table 1-3.

Treating the Patient and not the Platelet Count

ITP clinical specialists have increasingly advocated a less interventional approach to managing the disease.[73] This shift may be attributable to population-based findings of a low incidence of major bleeding events in patients with primary ITP[8,45] and to the side effects of long-term use of immunosuppressive therapies. In an observational study of 152 consecutive adults with primary ITP over a median follow-up time of 9.4 years (range: 0.2-22.6 years), for example, Portielje *et al.*[9] noted that more patients died from infection (N = 4) than bleeding (N = 2).

Accordingly, current treatment guidelines recommend against efforts to normalise platelet counts in adults and children with primary ITP but instead favour securing patient-specific platelet counts at which the bleeding risk is believed minimal.[2] Evidence suggests that patients may nevertheless continue to be excessively treated. Bolton-Maggs and Moon[74] uncovered a notable discrepancy between reported and recommended[75] practices in an audit of the management of paediatric primary ITP in the UK. Of a total of 427 cases evaluated, they documented inappropriate administration of IVIg in 86 children with clinically mild or asymptomatic disease. Additionally, while only 39 (26.2%) children with primary ITP exhibited wet bleeding at presentation, 353 (82.7%) had been hospitalised for a median duration of 3 days (range: 1-30 days). A large-scale audit of the management of primary ITP in adults has yet to be published though data suggest that guideline-discrepant practice is likely. In a retrospective, multi-centre investigation of 186 adults with primary ITP, for instance, Aledort *et al.*[76] observed that 87 (46.7%) patients had been diagnosed incidentally, yet 165 (88.7%) had been administered treatment. As Corrigan Jr.[77] aptly noted in an editorial accompanying Bolton-Maggs

and Moon's study, these data collectively indicate a continued tendency for "clinicians to be focusing on the platelet count and not the patient."

Quality of Life & Health-Related Lifestyle

Patients with primary ITP have historically been subject to considerable lifestyle restrictions, typically instigated by clinicians in the absence of data on disease progression. Despite the publication and dissemination of research highlighting a generally low risk of major haemorrhage, anecdotal reports patients and ITP clinical specialists suggest that potentially excessive limitations (*e.g.*, advice against airline travel and minimum-contact sports) persist. Investigation into the potential impact of these restrictions; of highly visible, if largely benign, bleeding manifestations; and of patient concerns over possible treatment side-effects[78] and often experienced fatigue[79] has only recently begun.

Emerging research in the field of health-related quality of life (HRQoL) has highlighted the considerable burden faced by patients with primary ITP. In a 2007 study, Zhou *et al.*[80] found significantly lower ($p < 0.01$) quality of life scores in 236 adults with primary ITP compared with 1,688 healthy adult controls across 6 of 8 evaluable categories[††††††] in the Short-Form (SF)-36, a non-disease specific, HRQoL questionnaire. Using the same instrument, McMillan *et al.*[81] further reported lower aggregate scores among 73 adults with primary ITP than in patients with hypertension, arthritis, or cancer. These investigations have coincided with successful efforts to develop and validate the ITP-Patient Assessment Questionnaire (ITP-PAQ)[82] and the Kids' ITP Tools (KIT),[83,84] instruments that will ultimately enable a more robust comparative evaluation of treatment modalities.

[††††††] The eight categories of the SF-36 are 1) physical functioning, 2) role limitations due to physical problems, 3) role limitations due to emotional problems, 4) body pain, 5) social functioning, 6) general health perception, 7) mental health, and 8) energy vitality. Zhou *et al.* noted statistically significant differences between adult patients with primary ITP and healthy adult controls in all but the latter two categories.

Thesis Objectives & Structure

While detailing the considerable progress that has been made since Harrington *et al.*'s foundational study, the aforementioned discussion further highlights unresolved questions concerning pathogenesis, treatment effectiveness, and co-morbid disease burden in adult patients with primary ITP. In an effort to collate the necessary materials to tackle these questions, I aimed to develop, initiate, and expand a National disease registry as part of my doctoral studies. This work was carried out under the supervision of Dr. Drew Provan, an internationally recognised adult ITP specialist at The Royal London Hospital.

Thesis Objectives

Data from the UK Adult ITP Registry, the Wellcome Trust Case-Control Consortium (WTCCC) 1958 British Birth Cohort, the GPRD, and peer-reviewed published studies were used to meet the following objectives of this thesis.

Disease Pathogenesis

1. To characterise associations of functional, candidate single nucleotide polymorphisms (SNPs) in cytokine or cytokine receptor genes with primary ITP in the Caucasian adult population.

Treatment Effectiveness

2. To assess the utility of autologous ^{111}Indium (^{111}In)-labelled platelet sequestration studies in patients with primary ITP prior to possible splenectomy.

3. To evaluate the effectiveness of eradication therapy in elevating platelet counts in *H. pylori*-infected, adult patients with primary ITP.

Co-Morbid Disease Burden

4. To document health-related lifestyle concerns among patients with primary ITP.

5. To characterise associations of primary ITP among adults with both arterial and venous thromboembolic events (TEs).

Thesis Outline

Chapter 2 describes the methodology of the establishment and operation of the UK Adult ITP Registry and the demographics, test results, and co-morbid disease prevalence of its participants at the time of diagnosis. **Chapter 3** details an investigation into the associations of 6 functional candidate SNPs in cytokine or cytokine receptor genes with primary ITP onset among Caucasian adults and with *H. pylori* infection status and disease severity in Caucasian adult patients with primary ITP. **Chapter 4** presents a retrospective study on the effectiveness of autologous ^{111}In-tropolone labelled platelet sequestration studies in predicting short, medium, and long-term outcomes from splenectomy in patients with primary ITP. **Chapter 5** reports a literature-based meta-analysis on the effects of eradication of *H. pylori* infection in patients with primary ITP. **Chapter 6** highlights health-related lifestyle concerns among patients with primary ITP. **Chapters 7 and 8** present studies on the relative risk of arterial and venous TEs in adults with and without primary ITP using data from the GPRD and the UK Adult ITP Registry, respectively. **Chapter 9** summarises the findings of the thesis, discusses the strengths and limitations of the analyses performed, and highlights ongoing research and promising avenues for future investigation. **Appendix 1** lists the publications that I authored during my doctoral studies. **Appendix 2** details the talks that I have given in conjunction with my work on the UK Adult ITP Registry and the projects that I have tackled with Dr. Provan to communicate research findings to patients with primary ITP. **Appendix 3** shows the health-related lifestyle questionnaire created by the ITP Support Association, and **Appendix 4** presents the proposal I authored to secure funding from GSK for the development of the UK Adult ITP Registry.

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Chapter 2: The development, initiation, and operation of the United Kingdom Adult ITP Registry

Summary

The United Kingdom Adult ITP Registry is an active, linked-anonymised, repository of longitudinal clinical data (demographics, bleeding events, ITP-specific treatments, laboratory results, and co-morbid conditions) and biological specimens (whole blood [~EDTA] or saliva [Oragene Saliva Kit]) on adults (≥ 16 years) with primary ITP. This chapter details the development and structure of the Registry, concluding with an overview of the baseline (i.e., at the time of diagnosis) demographics and co-morbid disease profile of its participants. By March 2010, comprehensive clinical data spanning a median post-diagnosis observation time of 5.6 years (inter-quartile range: 2.4-9.2 years) was available for 327 adults with primary ITP from 17 centres across the United Kingdom. The mean age of this cohort was 42.9 ± 19.2 years, and a female-to-male ratio of 1.7:1 was observed. Among patients for whom information was available, 41.5% had been secondarily referred (*i.e.*, referred to their enrolling centre by a haematologist and not a general practitioner), and 81.5% were Caucasian. The median baseline platelet count was 31 x 10^9/L (inter-quartile range: 9-80 x 10^9/L). These results are illustrative of a largely successful effort to launch a national registry, which now stands as one of the richest databases on primary ITP in the world.

Introduction

As discussed in Chapter 1, considerable questions remain with regard to disease pathogenesis, treatment effectiveness, and co-morbid burden in adults with primary ITP. Exploration of these queries requires a representative picture of patients with the disease and its clinical management over time. However, owing to the limited incidence (annual incidence: 1.6-2.4 per 100,000 adults)[1,2*] and prevalence (5-year period prevalence: 20.6-24.1 per 100,000 adults)[3,4] of the disease in the general adult population it is likely that the formation of such a picture will only follow the development of a registry or a systematic, multi-centre compilation of clinical data and biological material from adult patients with primary ITP.

History

Over the course of the 1990s, The Royal London Hospital emerged as the largest referral centre for adults with primary ITP in the UK. This growth was overseen by Professor Adrian Newland, who in the year 2000 recruited Dr. Drew Provan, then a haematology consultant at Southampton General Hospital, to direct and expand research into the disease. While a registry for children with primary ITP had already been initiated,† Dr. Provan observed that a similar registry for adults did not yet exist. He therefore spearheaded efforts for its creation, submitting to the London Research Ethics Committee (REC) in early 2002 a proposal for the "Establishment of a UK Registry for adults with idiopathic thrombocytopenic purpura (ITP) and investigation of the role of cytokine gene polymorphisms in the pathogenesis and natural history of autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura) in adults." The Registry was approved in August 2002 (London REC: 02/02/58) and was operational until June 2005, when Dr. Provan left The Royal London Hospital for a medical directorship with GlaxoSmithKline (GSK).

During this time, the Registry had been successfully populated with the names of 734 adults with primary ITP in the UK. This cohort was comprised of 332 consecutive patients from Barts and The London NHS Trust (*i.e.,* The Royal London Hospital or St. Bartholomew's Hospital) between January 1997 and June 2005 and 402 patients from collaborating hospitals between August 2002 and June 2005. Although blood samples (whole

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* When using a platelet count threshold of less than 50×10^9/L to define thrombocytopenia.

† The International Cooperative ITP Study Group, led by Professor Paul Imbach at the University Children's Hospital Basel UKBB (Basel, Switzerland) pioneered Registry work within the field of ITP and had by 2001 already conducted a comprehensive analysis on newly diagnosed children. See *Kuhne T, Imbach P, Bolton-Maggs PH, Berchtold W, Blanchette V, Buchanan GR. Newly diagnosed idiopathic thrombocytopenic purpura in childhood: an observational study. Lancet. 2001;358:2122-2125.*

blood, ~EDTA) had been secured from approximately 40% of these patients for subsequent DNA analyses, collation of clinical data on Registry participants had been extremely limited. Furthermore, work on the project had come to a virtual halt in Dr. Provan's absence. In July 2006, Dr. Provan returned to The Royal London Hospital and, with the aim of rekindling his research programme, was put in touch with me, a recently graduated M.Phil. student in epidemiology at the University of Cambridge, by Dr. Jamie Robinson, then head of oncology epidemiology at GSK.

Objective

Given the existing infrastructure and active encouragement from the ITP Support Association, Dr. Provan and I concurred that there was sufficient promise to seek to develop, initiate, and operate a revised Registry for adult patients with primary ITP in the UK.

Methods

Research Funding

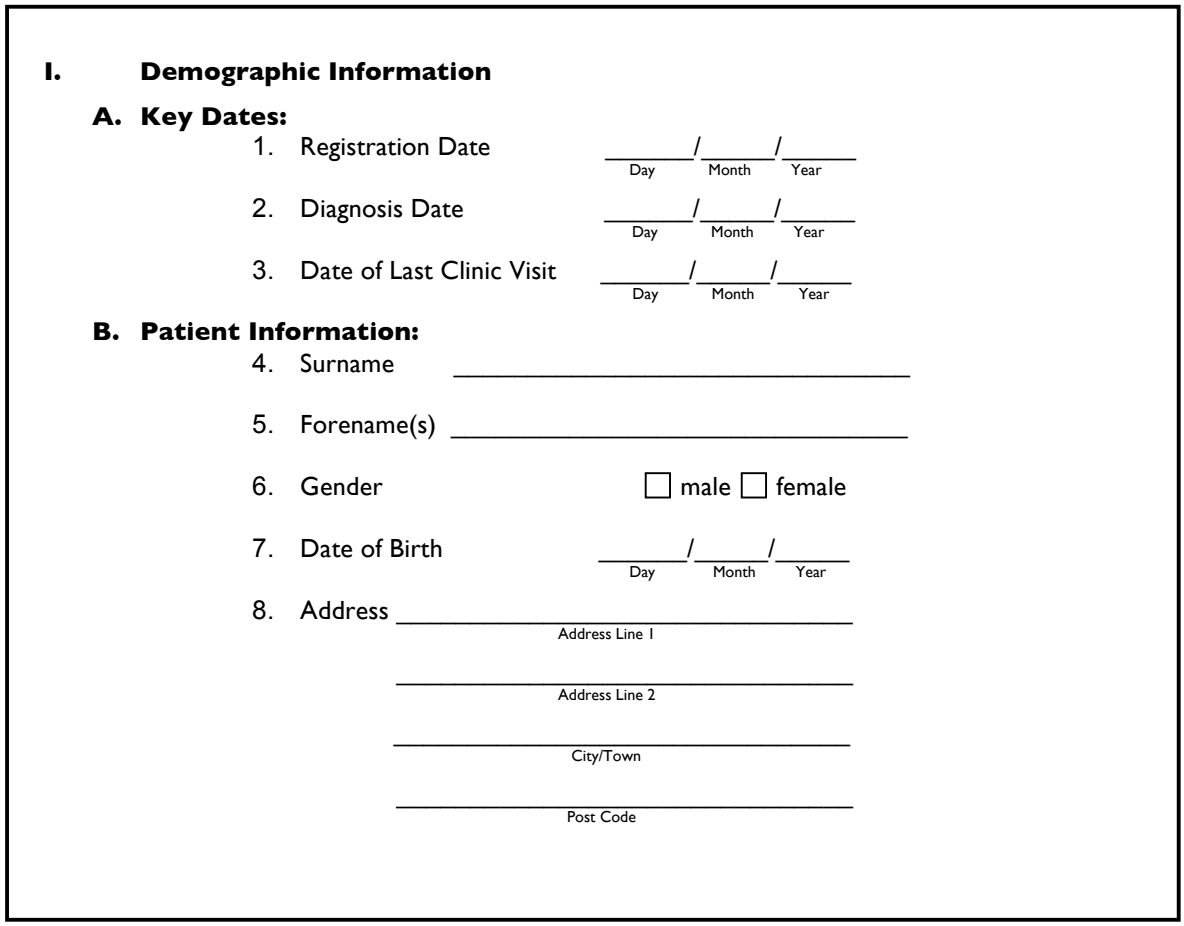
GSK was at this time nearing completion of a phase III trial of eltrombopag, an oral TPO receptor agonist, in adults with primary ITP. As interim results suggested that the drug was effective in stimulating platelet production in these patients,[5] the company was actively exploring how to fulfil its requirements for licensing approval from US and European regulators (*e.g.*, the Food and Drug Administration [FDA] and the European Medicines Agency [EMA], respectively). Recognising an overlap of interest in the timely collection of epidemiological information on primary ITP in adults, I drafted a grant proposal (Appendix 3) to secure the necessary resources to launch the revised Registry in exchange for the preparation of a series of reports on disease pathogenesis, treatment effectiveness, and co-morbid disease burden in adults with primary. Importantly, a stipulation was made that any agreement be predicated on written affirmation of Dr. Provan's continued ownership of Registry data and his autonomy over their use in published work.‡

‡ The benefits and drawbacks of our research having formal ties to a pharmaceutical company are discussed at length in Chapter 9.

Registry Structure

The revised Registry was structured to serve as a repository of biological material (15 mL whole blood [~EDTA] or saliva [Oragene Saliva Kit]) and longitudinal data on adults (≥ 16 years) who had been diagnosed with primary ITP in accordance with guidelines published by the British Committee for Standards in Haematology (BCSH).[6] Patients who had been diagnosed as children but in whom the disease persisted in adulthood were eligible for inclusion.

Clinical data collected for the Registry included demographics, bleeding events, ITP-specific treatments, co-morbid conditions, and laboratory test results. A full list of data fields captured is shown in the case report form (CRF) in Figure 2-1.



I. Demographic Information

A. Key Dates:

1. Registration Date _____/_____/_____ (Day / Month / Year)
2. Diagnosis Date _____/_____/_____ (Day / Month / Year)
3. Date of Last Clinic Visit _____/_____/_____ (Day / Month / Year)

B. Patient Information:

4. Surname _____
5. Forename(s) _____
6. Gender ☐ male ☐ female
7. Date of Birth _____/_____/_____ (Day / Month / Year)
8. Address _____ (Address Line 1)

_____ (Address Line 2)

_____ (City/Town)

_____ (Post Code)

Figure 2-1(a): Case Report Form-Part 1 of 6.

C. Hospital & Consultant Information:

9. Hospital Name _____

10. Departmental Address _____
Address Line 1

_____
Address Line 2

_____
City/Town

_____
Post Code

11. Consultant Name _____

II. Clinical Information

A. Patient Weight (Time of Diagnosis):

12. _____kg

B. Platelet Counts:

13.

Date (day/month/year)	Count ($\times 10^9$/L)

C. Bleeding Events:

	Location	Occurrence	Presentation Date(s)
14.	Cutaneous Bleeds	☐	____/____/____ ____/____/____
15.	Bleeds from the Oral Cavity	☐	____/____/____ ____/____/____
16.	Epistaxis	☐	____/____/____ ____/____/____
17.	Uterine Bleeds	☐	____/____/____ ____/____/____
18.	Haematuria	☐	____/____/____ ____/____/____
19.	Gastrointestinal Bleeds	☐	____/____/____ ____/____/____
20.	Intracranial Haemorrhage	☐	____/____/____ ____/____/____
21.	Muscle Bleeds	☐	____/____/____ ____/____/____
22.	Joint Bleeds	☐	____/____/____ ____/____/____
23.	Subconjunctival Bleeds	☐	____/____/____ ____/____/____
24.	Retinal Bleeds	☐	____/____/____ ____/____/____

Figure 2-1(b): Case Report Form-Part 2 of 6.

D. Treatments:

	Treatment	Administered	Date(s)	Dosage(s)	Course
25.	Prednisolone	☐	___/___/___ ___/___/___	_____ _____	_____ _____
26.	IVIg	☐	___/___/___ ___/___/___	_____ _____	_____ _____
27.	Splenectomy	☐ *Laparoscopic* ☐	___/___/___		
28.	Anti-D	☐	___/___/___ ___/___/___	_____ _____	_____ _____
29.	Methylprednisolone	☐	___/___/___ ___/___/___	_____ _____	_____ _____
30.	Dexamethasone	☐	___/___/___ ___/___/___	_____ _____	_____ _____
31.	Danazol	☐	___/___/___ ___/___/___	_____ _____	_____ _____
32.	Dapsone	☐	___/___/___ ___/___/___	_____ _____	_____ _____
33.	Azathioprine	☐	___/___/___ ___/___/___	_____ _____	_____ _____
34.	Cyclophosphamide	☐	___/___/___ ___/___/___	_____ _____	_____ _____
35.	Vinca Alkaloids	☐	___/___/___ ___/___/___	_____ _____	_____ _____
36.	Mycophenolate	☐	___/___/___ ___/___/___	_____ _____	_____ _____
37.	Plasmapheresis	☐	___/___/___ ___/___/___		
38.	Protein A Immunoadsorption	☐	___/___/___ ___/___/___		
39.	Interferon	☐	___/___/___ ___/___/___	_____ _____	_____ _____
40.	Cyclosporine	☐	___/___/___ ___/___/___	_____ _____	_____ _____
41.	Rituximab	☐	___/___/___ ___/___/___	_____ _____	_____ _____
42.	Platelet Transfusion	☐	___/___/___ ___/___/___	_____ _____	
43.	Red Blood Cell Transfusion	☐	___/___/___ ___/___/___	_____ _____	
44.	*H. pylori* Treatment	☐	___/___/___ ___/___/___		
45.	Vitamin C Supplements	☐	___/___/___ ___/___/___	_____ _____	_____ _____
46.	Other	☐	___/___/___ ___/___/___	_____ _____	_____ _____

Figure 2-1(c): Case Report Form-Part 3 of 6. Newer agents were evaluated alongside older therapies (*e.g.*, protein A immunoadsortion and interferon) for ITP.

E. Co-Morbid Conditions

	Condition	Occurrence	Diagnosis Date
47.	Cataracts	☐	____/____/____
48.	Osteoarthritis	☐	____/____/____
49.	Type-II Diabetes	☐	____/____/____
50.	Hypertension	☐	____/____/____
51.	Peptic Ulcers	☐	____/____/____
52.	*H. pylori* Infection	☐	____/____/____
53.	Renal Failure/Impairment	☐	____/____/____
54.	Chronic Liver Disease	☐	____/____/____
55.	Myocardial Infarction	☐	____/____/____
56.	Ischaemic Stroke	☐	____/____/____
57.	Transient Ischaemic Attack	☐	____/____/____
58.	Unstable Angina	☐	____/____/____
59.	Deep Vein Thrombosis	☐	____/____/____
60.	Pulmonary Embolism	☐	____/____/____
61.	Splenomegaly	☐	____/____/____
62.	Thyroid Disease	☐	____/____/____
63.	Depression/Anxiety	☐	____/____/____
64.	Miscarriage	☐	____/____/____
65.	Cushing's Syndrome	☐	____/____/____
66.	*Candida* Infection	☐	____/____/____
67.	Pneumonia	☐	____/____/____
68.	Other Autoimmune Disease	☐	____/____/____
69.	Haematological Malignancy	☐	____/____/____
70.	Solid Tumour/Malignancy	☐	____/____/____
71.	Photoxicity	☐	____/____/____

Figure 2-1(d): Case Report Form-Part 4 of 6. Recorded co-morbid conditions included commonly reported side effects of corticosteroids, thromboembolic events, and malignancies.

F. Biochemical Information (Time of Diagnosis): Serum Level

72. Alanine Transaminase (ALT) _____
73. Aspartate Transaminase (AST) _____
74. Alkaline Phosphatase (ALP) _____
75. Bilirubin _____

G. Haematological Information (Time of Diagnosis Except Where Noted):

		Performed	Result
76.	Blood Group		☐ A ☐ B ☐ A/B ☐ O Rh(D) ☐ + ☐ -
77.	Mean Platelet Volume (MPV)		_____
78.	Red Blood Cells (RBC)		_____
79.	White Blood Cells (WBC)		_____
80.	Haemoglobin (Hb)		_____
81.	Haemoglobin (Hb) [Last]		_____
82.	Neutrophils		_____
83.	Neutrophils [Last]		_____
84.	Direct Agglutination Test (DAT)	☐	☐ Positive ☐ Negative
85.	Marrow Aspirate	☐	Conclusion:_____
86.	Trephine Biopsy	☐	Conclusion:_____

H. Immunological Information (Time of Diagnosis)

		Performed	Serum Level
87.	IgG	☐	_____
88.	IgA	☐	_____
89.	IgM	☐	_____
90.	Anti-nuclear Antibodies (ANA)	☐	_____

Figure 2-1(e): Case Report Form-Part 5 of 6.

I. Coagulation Information (Time of Diagnosis)

		Performed	Serum Level/Percentage	Time/Ratio
91.	Prothrombin (PT) Ratio	☐		_____
92.	Activated Partial Prothrombin Time (APPT)	☐		_____
93.	Lupus Anticoagulant	☐	_____	
94.	IgG-Anticardiolipin Antibodies	☐	_____	
95.	IgM-Anticardiolipin Antibodies	☐	_____	
96.	Reticulocyte Percentage	☐	_____	

Figure 2-1(f): Case Report Form-Part 6 of 6.

As illustrated in Figure 2-2, data were retrospectively extracted at the time of patient registration and will be prospectively collected at annual intervals thereafter until censoring (*i.e.*, discharge from clinic or loss to follow-up) or Registry closure.

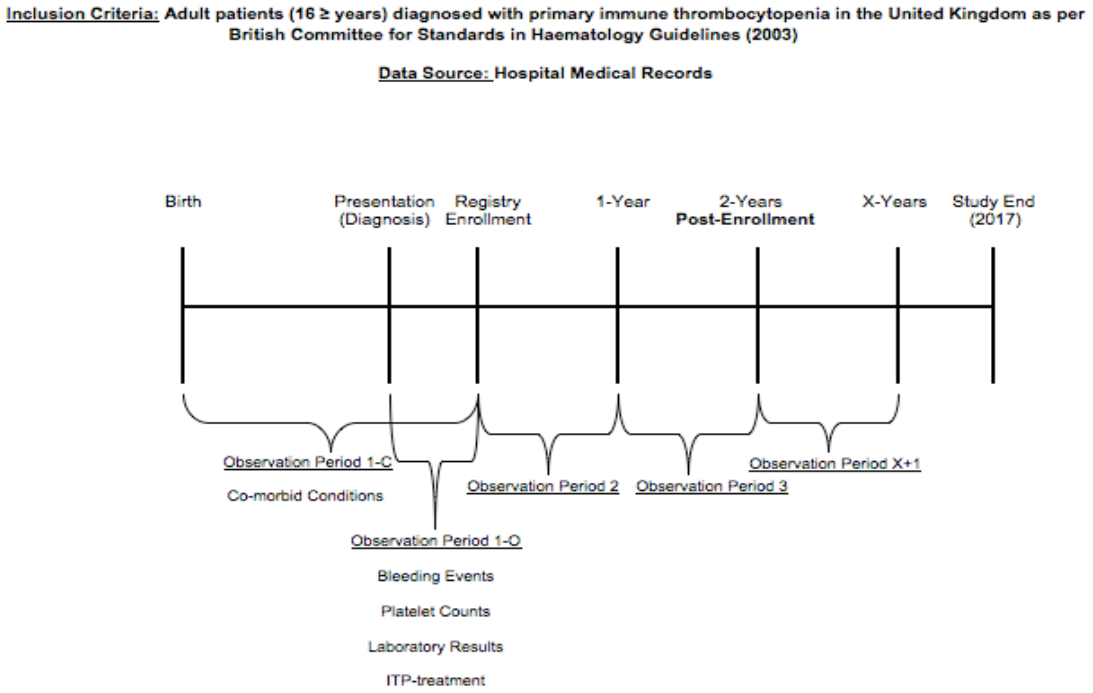


Figure 2-2: An overview of follow-up intervals in the UK Adult ITP Registry. At registration, a retrospective extraction of patient hospital medical records was performed for life-long co-morbidities, laboratory test results at diagnosis, and post-diagnosis bleeding events, platelet counts, and treatments. Prospective follow-up will be conducted at annual intervals thereafter.

Ethics

Applications for the approval to operate the aforestructured Registry were submitted to the Institute of Cell and Molecular Science (ICMS) at Barts and The London School of Medicine and Dentistry in March 2007, to the Joint Research Office at Barts and The London National Health Service (NHS) Trust in May 2007, and to the London REC in July 2007.

Patient Recruitment

A three-tiered strategy for recruitment was adopted, with a primary focus on securing comprehensive clinical data for past participants (*i.e.*, patients who had enrolled in the original Registry). Owing to substantive parallels between the original and revised Registries and the non-sensitive nature of proposed novel data collection, a request was made to the London REC for the automatic inclusion of past participants into the revised Registry. Written requests for clinical data were made to haematologists from collaborating centres while direct extraction of hospital medical records was performed by the Registry team at Barts and The London NHS Trust.

By late 2006, the Department of Nuclear Medicine at St. Bartholomew's Hospital remained one of only three centres in the UK to offer autologous ^{111}In-labelled platelet sequestration studies for patients with primary ITP. These studies are hypothesised to be an accurate predictor of the likelihood of success from splenectomy. Their efficacy has, however, been questioned by many ITP specialists, most prominently by Kojouri *et al.*[7] in a 2004 systematic review. To contribute to the body of evidence fuelling this ongoing debate, efforts were secondly made to enrol in the Registry all adult patients with primary ITP who had undergone testing since inception of the service in March 1994. Due to the combination of the size of this cohort and limited available resources, limited, splenectomy-specific data collection (Figure 2-3) was pursued for these patients.

Autologous ^{111}In-Labelled Platelet Sequestration Study Follow-Up Request
UK Adult ITP Registry

Dear Dr.

We hope that this message finds you well. Your patient with primary ITP underwent an Autologous ^{111}In-Labelled Platelet Sequestration Study prior to possible splenectomy. As part of an audit of our results, we are keen to know whether your patient had a splenectomy and, if so, what his/her platelet response following splenectomy was.

The data gathered over the course of this audit will be used to assess both the diagnostic precision of indium labelled platelet scanning and the utility of the extension of this technology to other centres. If possible, could you please complete this short form and fax or post it back to us? We thank you very much for your help on this request.

Patient name & DOB:	DOB needed:		
Consultant haematologist:			
Was a splenectomy performed?	**Yes/No**	**Date:**	
If no, please briefly give a reason:			
Pre-surgery-specific-treatment platelet count (×10⁹/L):		**Date:**	
Platelet count 1-3 months post post-surgery (×10⁹/L)		**Date:**	
Platelet count 6-12 months post-surgery (×10⁹/L)		**Date:**	
Latest platelet count (×10⁹/L):		**Date:**	
Was the patient receiving ITP-treatment at the time of the latest platelet count?	**Yes/No**		
If so, please specify:			

Yours Sincerely,

Dr Drew Provan
Chief Investigator & Consultant Haematologist

Mr Ameet Sarpatwari
Study Coordinator & Epidemiologist

Figure 2-3: Autologous ^{111}In-labelled platelet sequestration study follow-up form. Data collection was kept to a minimum to maximise response rates.

Thirdly, the Registry was opened to new patients throughout the UK. To streamline the process for hospitals to obtain local approval, applications were made for the Registry to be both site-specific assessment (SSA)-exempt and included in the United Kingdom Clinical Research Network (UKCRN) Portfolio Database, a collection of studies eligible for infrastructural support (*i.e.*, National Health Service [NHS] funding) from the National Institute for Health Research (NIHR). Advertisements for the launch of the Registry were made in *The Platelet*, the newsletter of the ITP Support Association and the *British Society for Haematology (BSH) Bulletin*; personal invitations to join the Registry were additionally sent to over 100 haematologists.

Data Collection

Extraction of hospital medical records for participants from Barts and The London NHS Trust were conducted by two-person teams led by trained epidemiologists. Physical records were first reviewed in full with the help of a research assistant.§ Data for extraction were tagged and orally relayed for direct entry into an electronic database, enabling cross-verification of recorded information. Catalogued clinical correspondence and electronic medical records were subsequently searched for additional data.

Collaborating centres were free to devise their own protocols, with data transfer taking place via paper-based submission or an encrypted, online database developed in partnership with Dendrite Clinical Systems.

§ Fourth or 5th-year medical or dental students from Barts and The London School of Medicine and Dentistry. A full list of past and present research assistants may be found at http://www.ukitpregistry.com/UKITPContactpage/page20/page20.html.

Results

Registry Development

Timelines for projects pertaining to the development and operation of the Registry are shown in Figure 2-4. Briefly, £267,875 in funding was secured from GSK in December 2006 and used to assemble a research team (*i.e.*, a data manager, a PhD student, and research assistants).** Extraction of hospital medical records at Barts and The London NHS Trust and active recruitment of collaborating centres were initiated following final ethical approval of the Registry an as SSA-exempt study in October 2007 (London REC: 07/H0718/57) and completion of the online database (http://host-dendrite.com/itpr) in November 2008, respectively. The Registry was adopted as a UKCRN Portfolio study†† in July 2008 and is scheduled to operate until October 2017. Data collection for the first series of analyses was concluded in March 2010.

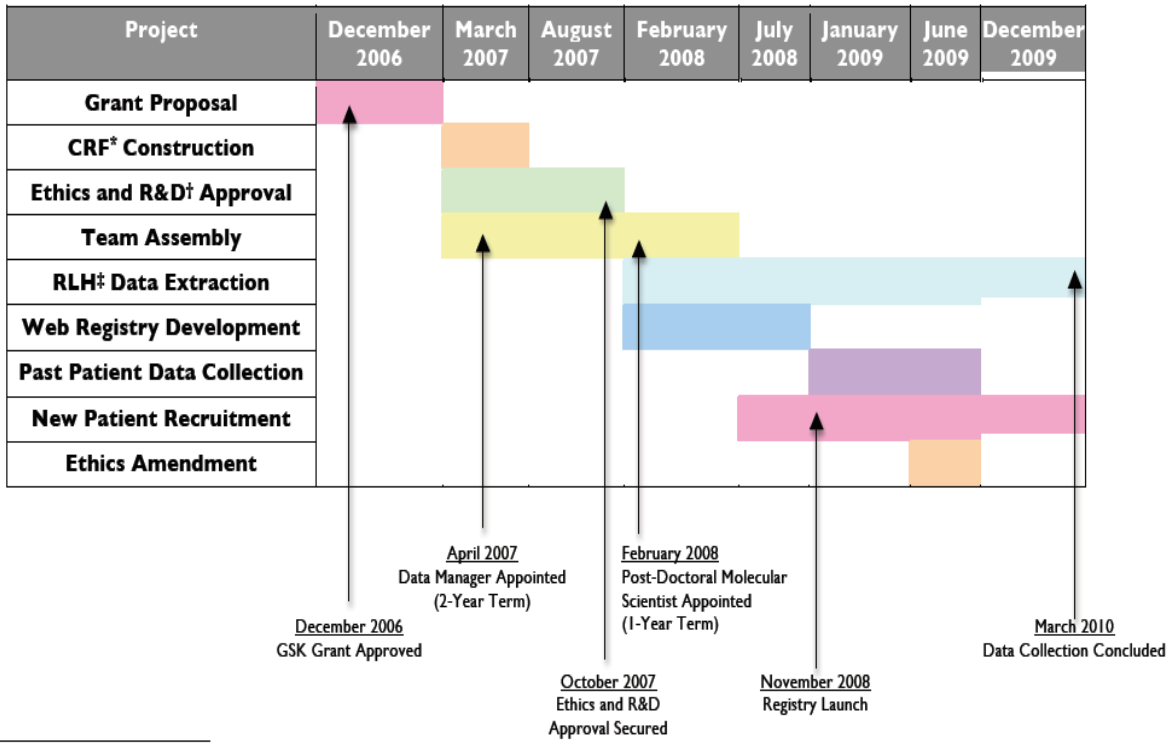


Figure 2-4: Project timeline and key events in the development of the UK Adult ITP Registry. The Registry was formally launched in November 2008 and will continue to enrol collaborating centres and participants until closure in October 2017.

** NB: The one-year molecular scientist post was funded by the ITP Support Association.
†† http://public.ukcrn.org.uk/Search/StudyDetail.aspx?StudyID=4961

Registry Centres and Participants

Four patient cohorts were assembled (Figure 2-5). During the extraction process it was noted that over half of the hospital medical records of Barts and The London patients not seen since 1999 had been lost or destroyed. A post-hoc decision was therefore made to restrict this cohort (Barts and The London Cohort) to consecutive adults with primary ITP attending haematology clinics between January 2000 and June 2005, resulting in a catchment population of 223 patients. These patients were coupled in the Registry with 104 patients from 16 collaborating NHS Trusts across the UK; 50 of these patients had been enrolled under the original Registry (Old External Cohort) and 54 under the revised Registry (New External Cohort). By January 2009, 265 patients with primary ITP had undergone autologous ^{111}In-labelled platelet sequestration studies at St. Bartholomew's Hospital (Autologous ^{111}In-Labelled Platelet Sequestration Study Cohort). As data collected on these patients were limited and used for splenectomy-related analyses alone, they were not included in the following description of Registry participants.

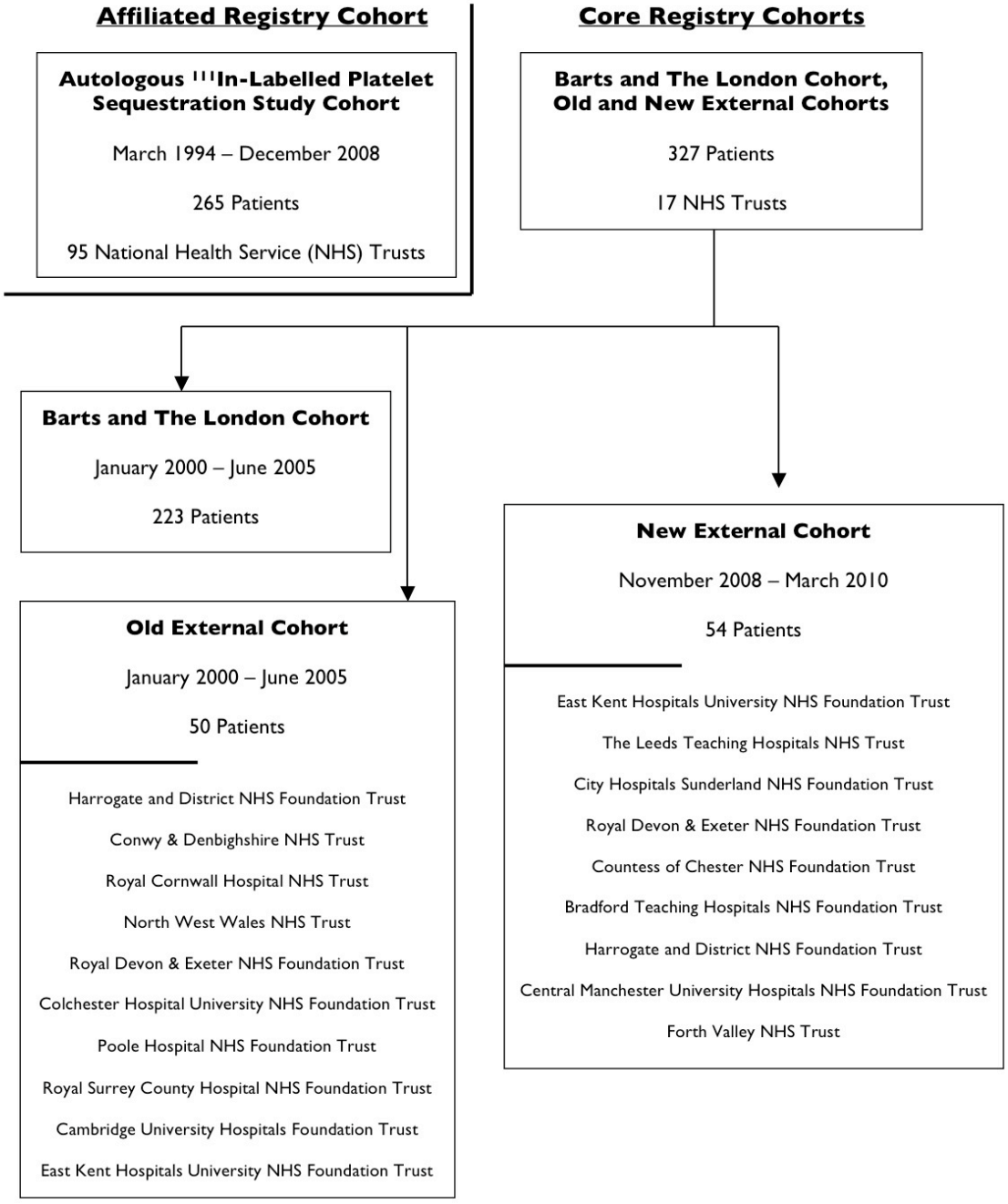


Figure 2-5: Overview of participant cohorts comprising the UK Adult ITP Registry. The core Registry entailed consecutive patients with primary ITP seen by a haematologist at Barts and The London NHS Trust between January 2000 and June 2005 (Barts and The London Cohort) and adults with primary ITP enrolled by collaborating centres under the original (Old External Cohort) and revised (New External Cohort) Registries.

In total, 327 adults with primary ITP were retrospectively followed for a median time of 5.6 years (inter-quartile range: 2.4-9.2 years). Table 2-1 details the baseline (*i.e.,* at the time of diagnosis) characteristics of the Registry population. The mean age of participants was 42.9 ± 19.2 years, and a female-to-male ratio of 1.7:1 was observed. Among patients for whom information was available, 41.5% had been secondarily referred (*i.e.*, referred to their enrolling centre by a haematologist and not a general practitioner), and 81.5% were Caucasian. The median baseline platelet count was 31 x 10^9/L (inter-quartile range: 9-80 x 10^9/L). Of 93 patients who were screened for anticardiolipin (IgG and IgM) and lupus anticoagulant antibodies at baseline, 18 (19.4%) tested positive for at least one of these classes of antiphospholipid antibodies.

Table 2-2 shows the baseline prevalence of co-morbid diseases among Registry participants. Prior TEs had been experienced by 19 patients (5.8%; 95% CI, 3.5%-8.9%), with 16 patients (4.9%; 95% CI, 2.8%-7.8%) having suffered an arterial TE and 4 (1.2%; 95% CI, 0.3%-3.1%) a venous TE.

A relatively a high percentage of patients (4.9%; 95% CI, 2.8%-7.8%) had a pre-existing or concurrently incident autoimmune disease at the time of primary ITP diagnosis, with 4 patients (1.2%; 95% CI, 0.3%-3.1%) meeting diagnostic criteria (*i.e.*, primary ITP and AIHA) for Evans syndrome. The true prevalence of autoimmune disease in the population was likely even higher. However, as autoantibody testing was not routinely performed in patients with hypo- or hyperthyroidism (3.1%; 95% CI, 1.5%-5.6% and 1.8%; 95% CI, 0.7%-4.0%; respectively), it was not possible to determine whether these conditions were autoimmune in nature.

Secondarily referred patients were on average younger (mean age: 36 ± 17 vs. 46 ± 19, $p < 0.001$) and more thrombocytopenic (mean count: 29 ± 33 vs. 64 ± 50, $p < 0.001$) at baseline than their primarily referred counterparts.

Table 2-1: Baseline Characteristics of Registry Participants‡‡

Variable	Number of Participants	Mean (SD) *or* Percentage
Socio-Demographic Variables		
Age at diagnosis (years)	318	42.9 (19.2)
Sex		
Female	206	63.0%
Male	121	37.0%
Haematologist referral		
No	144	44.0%
Yes	137	41.9%
Unknown	46	14.1%
Ethnicity		
Caucasian	207	81.5%
South Asian	27	10.6%
African	15	5.9%
Other	5	2.0%
Smoking status		
Never	88	63.8%
Current	25	18.1%
Former	25	18.1%
Haematological Variables		
Platelet count (/nl)	229	47 (46)
Red Blood Cell (RBC) count (/pL)	113	4.37 (0.73)
Haemoglobin (g/dL)	132	13 (2)
Neutrophil count (/nl)	100	5.0 (6.9)
White Blood Cell (WBC) count (/nL)	125	6.9 (2.7)
Immunological Variables		
Total IgG (mg/dL)	80	1264 (514)
Total IgA (mg/dL)	80	224 (105)
Total IgM (mg/dL)	80	165 (191)
Anticardiolipin Antibody-IgG	102	
Negative	96	94.1%
Positive	6	5.9%
Anticardiolipin Antibody-IgM	102	
Negative	97	95.1%
Positive	4	3.9%
Borderline	1	1.0%
Lupus Anticoagulant	95	
Negative	84	88.4%
Positive	10	10.5%
Borderline	1	1.1%
Indium Scanning-Sequestration Pattern	57	
Mixed or Hepatic	33	57.9%
Purely or Predominantly Splenic	24	42.1%
H. pylori IgG antibody	36	
Negative	18	50.0%
Positive	16	44.4%
Equivocal	2	5.6%
Biochemical Variables		
Total Bilirubin (μmol/L)	110	11 (9)
ALT (U/L)	106	25 (20)
AST (U/L)	99	26 (16)

‡‡ Data involves up to 327 participants.

Table 2-2: Baseline Prevalence of Co-morbid Conditions[§§]

Co-Morbid Disease	Number of Participants	Percentage (95% CI[***])
Hypertension	23	7.0 (4.5-10.4)
Autoimmune diseases[‡]	16	4.9 (2.8-7.8)
Autoimmune Haemolytic Anaemia	4	1.2 (0.3-3.1)
Reynaud's phenomenon	2	0.6 (0.1-2.2)
Rheumatoid arthritis	2	0.6 (0.1-2.2)
Autoimmune cytopenia	2	0.6 (0.1-2.2)
Eczema	2	0.6 (0.1-2.2)
Inflammatory bowel disease	1	0.3 (0-1.7)
Multiple Sclerosis	1	0.3 (0-1.7)
Psoriasis	1	0.3 (0-1.7)
Hypothyroidism[‡]	10	3.1 (1.5-5.6)
Renal disease	9	2.8 (1.3-5.2)
Miscarriage[†††]	8	3.9 (1.7-7.5)
Osteoarthritis	8	2.4 (1.1-4.8)
Type 2 diabetes	8	2.4 (1.1-4.8)
Solid tumour	8	2.4 (1.1-4.8)
Myocardial infarction (MI)	6	1.8 (0.7-4.0)
Unstable angina (UA)	6	1.8 (0.7-4.0)
Hyperthyroidism[‡‡‡]	6	1.8 (0.7-4.0)
Ischaemic stroke (IS)	5	1.5 (0.5-3.5)
Transient ischaemic attack (TIA)	4	1.2 (0.3-3.1)
Candidiasis	4	1.2 (0.3-3.1)
Long-bone fracture	3	0.9 (0.2-2.7)
Deep vein thrombosis (DVT)	3	0.9 (0.2-2.7)
Cataracts	2	0.6 (0.1-2.2)
Liver disease	2	0.6 (0.1-2.2)
Haematological malignancy	2	0.6 (0.1-2.2)
Pulmonary embolism (PE)	2	0.6 (0.1-2.2)
Peptic ulcer disease	1	0.3 (0-1.7)

[§§] Data involves up to 327 participants.
[***] Confidence interval
[†††] The denominator is the total number of female participants (N = 206).
[‡‡‡] It was not possible to identify autoimmune thyroid diseases as antibody testing was not regularly performed for patients with thyroid disorders.

Discussion

The aforementioned results highlight a generally successful effort to develop and launch a national registry for adults with primary ITP. Multiple stumbling blocks were admittedly encountered during this process, including limited functionality of the online database. Data entry and site navigation were not straightforward, and specifications for an annual follow-up reporting system were never implemented by the hosting company.

Due in part to these limitations, fewer patients from collaborating centres were enrolled than had been targeted (104 patients enrolled vs. 200 patients targeted). The Registry nevertheless emerged as one of the largest and most comprehensive repositories of clinical data on adults with primary ITP in the world. I, moreover, endeavoured to learn from these missteps, having more explicitly discussed the needs and expectations of our research team in March 2010 in hiring a new data manager and contracting for the development of a new online database with Medical Data Solutions and Services (MDSAS), a company recently hired by the Department of Health to replace Dendrite in building and operating a National Immunoglobulin Database.

The limited number of patients enrolled from a subset of centres raises the spectre of selection bias, namely the possibility that patients who were enrolled were systematically different (*e.g.*, had greater disease severity) than those were not. However, post-hoc subgroup analyses of baseline participant characteristics revealed that the mean age and platelet count did not differ significantly between participants from centres enrolling more than 3 patients and centres enrolling 3 or fewer patients (Table 2-3), suggesting that such bias was minimal, if present.

Table 2-3: Subgroup Analyses of Baseline Data

Variable	Centres Enrolling ≤ 3 Patients		Centres Enrolling > 3 Patients		P-Value
	Number	Mean (SD) or %	Number	Mean (SD) or %	
Age (Years)	23	53.2 (18.8)	79	50.9 (18.5)	0.56
Platelet Count ($\times 10^9$/L)	11	33.5 (13.5)	46	37.7 (5.5)	0.21
Female	12	52.2%	49	60.9%	0.48

The percentage of South Asians in the Registry, 10.6%, was less than initially expected. Patients from The Royal London Hospital comprised 68.2% of the Registry population,[§§§] and its catchment area, the borough of Tower Hamlets, is 30% Bangladeshi.[****] Given these figures, one would expect roughly 20% of the Registry to be South Asian.[††††] This apparent discrepancy may be explained by relative age distribution within the Bangladeshi and Caucasian populations. Only 14% of Caucasians in Tower Hamlets are between the ages of 0 and 15 years; this figure is, however, 35% among Bangladeshis.[****] Therefore, the data likely do not implicate an ethnic disparity in the prevalence of primary ITP.

While primarily referred Registry participants may well be representative of the general population of adults with primary ITP, secondarily referred participants are likely more reflective of patients with severe disease. This assumption is supported by baseline data, which revealed a significantly lower mean age (36 ± 17 years vs. 46 ± 19, $p < 0.001$) and platelet count ($29 \pm 33 \times 10^9$/L vs. $64 \pm 50 \times 10^9$/L, $p < 0.001$) among secondarily referred participants. The external validity of analyses from Registry data, which couples information from primarily and secondarily referred participants, may therefore be limited to the primary ITP population typically encountered at referral centres (*i.e.*, not district hospitals).

In summary, efforts to develop, initiate, and operate the UK Adult ITP Registry have yielded sufficient data to begin addressing unresolved questions of pathogenesis, treatment effectiveness, and co-morbid disease burden among adults with primary ITP. However, considerable room for growth remains. Such expansion will ultimately be needed to resolve these questions definitively.

[§§§] NB: Almost all of the Registry participants from Barts and The London NHS Trust were patients at The Royal London Hospital and not St. Bartholomew's Hospital.

[****] Tower Hamlets Council. "Ethnicity." *Tower Hamlets*. 28 May 2010 http://www.towerhamlets.gov.uk/lgsl/901-950/916_borough_statistics/ethnicity.aspx.

[††††] 327 (total patients) × 0.682 (proportion of patients from The Royal London Hospital) × 0.3 (approximate proportion of Bangladeshi patients at The Royal London Hospital) ≈ 65 patients ≈ 20%.

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Chapter 3: Associations of functional candidate single nucleotide polymorphisms (SNPs) in cytokine or cytokine receptor genes with primary ITP in Caucasian adults

Summary

Although T-helper (Th)1 polarisation has been well documented in patients with primary ITP, the genetic contribution to this imbalance remains unclear. To address this question, 6 functional, candidate single nucleotide polymorphisms (SNPs) within cytokine or cytokine receptor genes were selected for association testing with primary ITP in Caucasian adults. Patients from the United Kingdom Adult ITP Registry were sex-matched (1:3) with healthy controls from the Wellcome Trust Case-Control Consortium 1958 British Birth Cohort. Variants *IL10* -819 c>t, *TNFA* -308 g>a, *TGFB1* -509 c>t, *IL1A* -889 c>t, *IL10* -592 c>t, and *IL4R* q576r were measured in samples from cases and retrieved from the European Genome-phenome Archive for controls. Associations were evaluated using logistic regression models. Overall, 206 patients with primary ITP were matched with 618 healthy controls. A significant, per allele odds ratio of 1.34 (95% CI, 1.03-1.75) was observed for *TNFA* -308 g>a, implicating increased disease susceptibility among Caucasian carriers of the rare allele.

Introduction

As discussed in Chapter 1, the traditional Mosmann-Coffman[1] model for the classification of Th cells remains a useful construct from which to evaluate the pathophysiology of autoimmune disease. Under this paradigm, homeostasis is maintained through the balance of cytokines secreted by Th1 (*e.g.*, interferon [IFN]-γ, IL-2, tumor necrosis factor [TNF]-α, and TNF-β1) and Th2 (*e.g.*, IL-4, Il-5, IL-6, IL-10,* and IL-13) cells.[2] While the former promote pro-inflammatory, cell-mediated and complement-fixing IgG isotype (IgG3 & IgG1) responses effective in the combat of intracellular pathogens, the latter elicit immediate-type hypersensitivity, augmenting humoral defense against extracellular pathogens.[3,4] Crucially, Th1 and Th2 cell responses further demonstrate down regulatory effects on each other.[5]

Although past proteomic investigations have demonstrated Th1 polarisation in both adults and children with primary ITP,[6-8] questions remain concerning the genetic contribution to this imbalance. Recent studies have highlighted a non-random clustering of susceptibility loci for clinically distinct autoimmune diseases,[9,10] implicating the existence of common susceptibility gene variants. However, few investigations have assessed the relationship of primary ITP with such polymorphisms, including copy number variations (CNVs), human leukocyte antigen (HLA) alleles, and SNPs.[11-29] These studies are summarised in Tables 1 and 2. To enable comparisons of results across SNP and bi-allelic CNV studies, raw data from published reports were extracted and re-expressed as per rare allele odds ratios (ORs) with 95% CIs using logistic regression models. Pearson's chi-square tests were performed for comparisons of observed and expected genotype frequencies under Hardy-Weinberg equilibrium (HWE). Owing to the large number of alleles evaluated, only significant (α = 0.05) results† were reported for HLA investigations. Importantly, no corrections were made for multiple testing.

As shown in Table 1, half of all studies among adults with primary ITP evaluated cohorts of less than 100 patients. Furthermore, of these investigations, only 4[15,17,21,22] assessed SNPs within cytokine or cytokine receptor genes, all among non-Caucasian populations. The primary objective of our study, therefore, was to test for associations of functional, candidate SNPs in cytokine or cytokine receptor genes with primary ITP among Caucasian adults.

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*As IL-10 is produced by both Th1 and Th2 cells, disagreement exists as to whether it can be properly classified as a Th2 cytokine.

†As determined by Pearson's chi-square or Fisher's exact tests.

Table 3-1(a): Genetic Variant Studies among Adults with Primary ITP (1998-2007)

Author & Year	Participant Ethnicity	Primary ITP Cohort Restrictions Beyond 1997 ASH* Criteria	Primary ITP Cohort-*N*	Primary ITP-Free Cohort-*N*	Gene(s) Evaluated	SNP, HLA, or CNV‡ Alleles Evaluated	Results *Not Corrected for Multiple Testing*	HWE‡ (P-Value)
Nomura *et al.* 1998	Japanese	•Bone marrow aspirate	111	71	*HLA-DRB1*	401, 403-0408, 410, 802-804, 901	*HLA-DRB1*0401*: p < 0.01	N/A
Kuwana *et al.* 2000	Japanese	•TBC‡: < 50 x 10⁹/L •Bone marrow aspirate	83	114	*HLA-DPB1* *HLA-DQB1* *HLA-DRB1, -DRB3, -DRB4*	DPB1-201, 202, 301, 401, 402, 501, 601, 901, 1301, 1401, 1901; DQB1-201, 301-303, 401, 402, 501-503, 601-604; DRB1-101, 401, 403-407, 410, 802, 803, 901, 1001, 1101, 1102, 1201, 1202, 1301, 1302, 1401-1403, 1405, 1407, 1501, 1502, 1602; DRB3-101; 202; 301; DRB4-103	*HLA-DPB1*0201*: p < 0.01 *HLA-DQB1*0501*: p = 0.01 *HLA-DRB1*0101*: p = 0.01	N/A N/A N/A
Fujimoto *et al.* 2001	Japanese	•TBC: < 100 x 10⁹/L •Bone marrow aspirate	104	59	*FCGR2A* *FCGR3A*	+131 h>r; rs1801274 +158 v>f; rs396991	1.18 (0.70-1.98) 1.72 (1.00-2.99)	0.50 0.21
Stanworth *et al.* 2001	Caucasian	•Chronic: *not defined* •Bone marrow aspirate	71	750	*HLA-A* *HLA-B* *HLA-DRB1*	01, 02 08 03, 04, 13	*HLA-A*02*: p = 0.03	N/A
Satoh *et al.* 2004	Japanese	•TBC: < 100 x 10⁹/L •Bone marrow aspirate	84	56	*IL1B* *TNFA* *TNFB1/LTA* *HLA-DRB1, -DQB1, -DPB1*	-511 c>t; rs16944 +3953 t>c; rs1143634 -238 g>a; rs361525 -308 g>a; rs1800629 +252 g>a; rs909253 *Data not provided*	1.32 (084-2.07) 0.82 (0.21-3.21) 0.21 (0.02-2.10) p = 0.10† 0.55 (0.33-0.91) N/A	0.45 0.78 0.84 N/A 0.85 N/A
Veneri *et al.* 2005	Caucasian	N/A	52	N/A‡	*HLA-DQB1* *HLA-DRB1*	02, 03, 04, 05, 06 1, 3, 4, 7, 8, 910, 1001, 11, 12, 13, 14, 15, 16	*HLA-DQB1*03*: p = 0.04 *HLA-DRB1*03*: p = 0.05	N/A N/A
Chen *et al.* 2007	Han-Chinese	•TBC: < 100 x 10⁹/L	121	128	*IFNG* *IL4*	+874 a>t; rs243056 CNV in intron 3; rp1>rp2	ITP: 1.33 (0.78-2.27) Acute: 0.58 (0.20-1.69) ITP: 0.88 (0.57-1.37) Acute: 1.04 (0.46-2.34)	0.63 0.19 0.74 0.58

* ASH: American Society of Hematology; CNV: copy number variation; HLA: human leukocyte antigen; HWE: Hardy-Weinberg equilibrium; SNP: single nucleotide polymorphism; TBC: thrombocytopenia
†Insufficient rare allele frequency prohibited logistic regression modelling; a 3x2 Fisher's exact test was performed instead.
‡ *H. pylori*-positive vs. *H. pylori*-negative patients & responders vs. non-responders to eradication therapy

Table 3-1(b): Genetic Variant Studies among Adults with Primary ITP (2008-Present)

Author & Year	Participant Ethnicity	Primary ITP Cohort Restrictions Beyond 1997 ASH Criteria	Primary ITP Cohort-*N*	Primary ITP-Free Cohort-*N*	Gene(s) Evaluated	SNP, HLA, or CNV Alleles Evaluated	Results *Not Corrected for Multiple Testing*	HWE (P-Value)
Breunis *et al.* 2008	Caucasian	N/A	44	100	*FCGR2A*	+131 h>r; rs1801274	0.93 (0.55-1.57)	0.64
					FCGR2B	+232 i>t; rs1050501	0.88 (0.51-1.51)	0.01
					FCGR2C	-386 g>c; rs3219018	2.36 (1.14-4.85)	0.15
						CNV in exon 3; 1/2/3 copies§	1.22 (0.48-3.09)	N/A
					FCGR3A	CNV in intron 1; 1/2/3 copies	1.04 (0.25-4.32)	N/A
					FCGR3B	CNV in intron 1; 1/2/3 copies	1.22 (0.48-3.09)	N/A
Chen *et al.* 2008	Han-Chinese	•TBC: < 100 x 10^9/L	119	136	*DNMT3B*	c46359t; rs2424913	ITP: 1.15 (0.23-5.79) Acute: 1.98 (0.17-22.78)	0.90 0.92
Xu *et al.* 2008	Han-Chinese	•TBC: < 100 x 10^9/L	118	169	*CD72*	CNV in intron 8; 1>2	1.41 (1.02-1.93)	0.27
Suzuki *et al.* 2008	Japanese	•*H. pylori* positive	36	N/A**	*IL1B*	-31 t>c; rs1143627	0.71 (0.30-1.69)	0.32
					IL1RN	CNV in intron 2; l>s	0.53 (0.10-2.81)	0.57
					TNFA	-308 g>a; rs1800629	p = 0.15††	N/A††
					TNFB1/LTA	+252 g>a; rs909253	0.48 (0.20-1.14)	0.02
Satoh *et al.* 2009	Japanese	•Chronic: ≥ 0.5 years	164	75	*IL1B*	-511 c>t; rs16944	ITP: 1.02 (0.71-1.47) *H. pylori*: 0.80 (0.53-1.20)	0.59 0.91
			H. pylori (+) 85		*TNFB*	+252 g>a; rs361525	ITP: 1.18 (0.80-1.72) *H. pylori*: 1.13 (0.72-1.78)	0.02 0.69
			H. pylori (-) 79		*IgG1 (Heavy Chain)*	+643 g>a; *unknown rsnumber*	ITP: 1.70 (0.92-3.11) *H. pylori*: 1.05 (0.51-2.17)	0.59 0.81
					IgK	+573 c>g; *unknown rsnumber*	ITP: 1.15 (0.72-1.84) *H. pylori*: 0.93 (0.57-1.52)	0.21 0.93

§ CNV results for Breunis *et al.* are expressed as per rare copy effect.
** Responders v. non-responders to *H. pylori* eradication therapy
†† No a alleles observed.

Table 3-2(a): Genetic Variant Studies among Children‡‡ with Primary ITP (2001-2004)

Author & Year	Participant Ethnicity	Primary ITP Cohort Restrictions Beyond 1997 ASH Criteria	Primary ITP Cohort-*N*	Primary ITP-Free Cohort-*N*	Gene(s) Evaluated	SNP, HLA, or CNV Alleles Evaluated	Results *Not Corrected for Multiple Testing*	HWE (P-Value)
Williams *et al.* 1998	Caucasian	•Splenectomised	29	61	*FCGR2A*	+131 h>r; rs1801274	2.81 (1.35-5.83)	0.23
Foster *et al.* 2001	Caucasian	•Chronic: ≥ 0.5 years	37	218	*IL1A*	-889 c>t; rs1800587	0.57 (0.31-1.06)	0.29
					IL1B	+3953 t>c; rs1143634	0.90 (0.52-1.58)	0.08
					IL4	-590 g>t; rs2243250	0.76 (0.37-1.55)	0.07
					IL6	-174 c>g; rs1800795	0.98 (0.58-1.64)	0.23
					IL10	-1082 a>g; rs1800896	1.36 (0.80-2.31)	0.16
						-592 a>c; rs1800872	0.89 (0.47-1.67)	0.64
					ILRN	CNV in intron 2; l>s §§	0.72 (0.41-1.29)	0.13
					TNFA	-308 g>a; rs1800629	0.40 (0.15-1.07)	0.02
					TNFB1/LTA	+252 g>a; rs909253	1.55 (0.96-2.47)	0.01
					FCGR2A	+131 h>r; rs1801274	1.42 (0.87-2.31)	0.11
					FCGR3A	+158 v>f; rs396991	1.12 (0.65-1.90)	0.47
					FCGR3B	na1>na2; N/A	0.52 (0.31-0.87)	0.39
Carcao *et al.* 2003	Caucasian	N/A	93	130	*FCGR2A*	+131 h>r; rs1801274	1.51 (1.03-2.21)	0.48
					FCGR3A	+158 v>f; rs396991	0.60 (0.40-0.90)	0.81
Pavkovic *et al.* 2003	Caucasian	N/A	60	100	*CTLA4*	+49 a>g; rs231775	1.23 (0.76-1.98)	0.53
Bruin *et al.* 2004	Caucasian	•TBC: < 50 x 10^9/L	60	N/A***	*FCGR2A*	+131 h>r; rs1801274	0.93 (0.40-2.15)	0.63
					FCGR2B	+232 i>t; rs1050501	0.16 (0.26-1.00)	0.57
					FCGR3A	+158 v>f; rs396991	1.35 (0.57-3.19)	0.78
					FCGR3B	na1>na2; N/A	1.04 (0.44-2.46)	0.50

‡‡ Or combined results among adults and children with primary ITP that were unable to be separated

Table 3-2(b): Genetic Variant Studies among Children with Primary ITP (2005-Present)

Author & Year	Participant Ethnicity	Primary ITP Cohort Restrictions Beyond 1997 ASH Criteria	Primary ITP Cohort-*N*	Primary ITP-Free Cohort-*N*	Gene(s) Evaluated	SNP, HLA, or CNV Alleles Evaluated	Results *Not Corrected for Multiple Testing*	HWE (P-Value)
Wu *et al.* 2005	Han-Chinese	•Bone marrow aspirate	80	100	*IL4*	CNV in intron 3; rp1>rp2	ITP: 0.81 (0.47-1.38) Acute: 3.45 (1.15-10.40)	0.61 0.70
					IL6	-572 g>c; rs1800796	ITP: 1.28 (0.81-2.00) Acute: 1.14 (0.60-2.20)	0.79 0.90
					IL10	-627 c>a; rs1800872	ITP: 0.80 (0.53-1.21) Acute: 0.88 (0.48-1.64)	0.57 < 0.01
Wu *et al.* 2007	Han-Chinese	•Bone marrow aspirate	80	100	*IL1B*	+3953 t>c; rs1143634	ITP: 1.44 (0.47-4.44) Acute: 1.22 (0.21-7.08)	< 0.01 0.85
					IL1RN	CNV in intron 2; 1>2	ITP: 0.32 (0.10-1.02) Acute†††; p = 0.02	0.45 0.70
Chen *et al.* 2007	Han-Chinese	•TBC: < 100x10⁹/L	75	128	*IFNG*	+874 a>t; rs243056	ITP: 1.27 (0.68-2.37) Acute: 0.60 (0.13-2.86)	0.63 0.78
					IL4	CNV in intron 3; rp1>rp2	ITP: 0.84 (0.51-1.38) Acute: 1.30 (0.42-4.06)	0.74 0.31
Breunis *et al.* 2008	Caucasian	N/A	72	100	*FCGR2A*	+131 h>r; rs1801274	1.01 (0.66-1.53)	0.64
					FCGR2B	+232 i>t; rs1050501	0.86 (0.46-1.62)	0.01
					FCGR2C	-386 g>c; rs3219018 CNV in exon 3; 1/2/3 copies‡‡‡	2.02 (1.05-3.89) 0.66 (0.30-1.41)	0.15 N/A
					FCGR3A	CNV in intron 1; 1/2/3 copies	0.41 (0.78-2.15)	N/A
					FCGR3B	CNV in intron 1; 1/2/3 copies	0.66 (0.30-1.41)	N/A
Chen *et al.* 2008	Han-Chinese	•TBC: < 100x10⁹/L	82	136	*DNMT3B*	c46359t; rs2424913	ITP: 2.27 (0.50-10.42) Acute: 1.83 (0.18-19.13)	0.90 0.85
Xu *et al.* 2008	Han-Chinese	•TBC: < 100x10⁹/L	88	169	*CD72*	CNV in intron 8; 1>2	ITP: 0.81 (0.56-1.15) Acute: 1.07 (0.51-2.22)	< 0.01 0.26

§§ CNV results for Foster *et al.* reported using rubric put forward by Suzuki *et al.* (*i.e.*, short vs. long alleles).
*** Chronic vs. non-chronic TBC
††† Insufficient rare allele frequency prohibited logistic regression modelling
‡‡‡ CNV results for Breunis *et al.* are expressed as per rare copy effect

Methods

Study Design and Selection of Candidate SNPs

A case-control design was adopted and 6 functional SNPs within cytokine or cytokine receptor genes selected on the basis of their associations with other autoimmune diseases in the medical literature. They are described briefly below

IL10 -819 c>t (rs1800872) & -592 c>t (rs1800871)

IL-10 is an anti-inflammatory cytokine that inhibits Th1 cytokine production while promoting B cell differentiation.[30] Both -819 c>t (rs1800871) and -592 c>t (rs1800871) have been implicated as functional SNPs. Turner *et al.*[31] and Crilly *et al.*[32] have documented decreased IL-10 production in individuals with the rare allele haplotype, ATA (SNPs: -1082 g>a, -819 c>t, -592 c>a), Associations of this haplotype with SLE[33] and rheumatoid arthritis[34] have been highlighted in the medical literature.

TNFA -308 g>a (rs1800629)

TNF-α is a pro-inflammatory cytokine with powerful cytotoxic properties.[35] The *TNF-A* gene is located in the HLA class III region between the HLA-B and HLA-DRB3 loci in 6p21.3.[36] A number of polymorphisms have been described within its promoter region, including -308 g>a (rs1800629). The rare allele of this SNP may be tied to increased TNF-α levels[37] and is increased in frequency among patients with rheumatoid arthritis,[38] SLE,[39] inflammatory bowel disease,[40] and coeliac disease.[41]

TGFB1 -509 c>t (rs1800469)

TGF-β1 is a dimeric cytokine that inhibits lymphocyte proliferation and activation.[42] The *TGFB1* gene is located in 19q13, and 3 SNPs have been reported in its promoter region. Of these, -509 c>t (rs1800469) has been shown to be functionally relevant, with common allele homozygotes exhibiting roughly half the plasma levels of TGF-β1 as rare allele homozygotes.[43] The SNP has recently been associated with chronic idiopathic neutropenia.[44]

IL1A -889 c>t (rs1800587)

The *IL1A* gene is one of three members of the IL-1 family (*IL1A, IL1B, ILRN*) located in 2q14. IL-1α and IL-1β are pro-inflammatory cytokines involved in a wide array of host organism defences against infection and compete with IL-1RA, a non-signalling anti-inflammatory molecule encoded by *ILRN*.[45] Homozygotes for the IL1A -889 c>t (rs1800587) rare allele exhibit increased mRNA and protein levels of IL-1α relative to their common allele counterparts.[46] The SNP has been reported to be associated with SLE[47] and psoriatic arthritis.[48]

IL4R q576r (rs1801275)

IL-4 is an anti-inflammatory cytokine that plays a key role in humoral immunity though the induction of naïve T cell differentiation into Th2 cells.[49] The IL-4 receptor (IL-4R) is a heterodimeric complex consisting of an α and common γ chain.[49] In the SNP +1727 a>g (rs1801275), the rare allele results in the substitution of glutamine with arginine at codon 551, leading to increased receptor activity.[50] Associations of the SNP have been found with atopic disorders,[50] SLE, and type I diabetes.[51]

Study Subjects

Primary ITP cases, as defined by 2003 British Committee for Standards in Haematology (BCSH) guidelines,[52] comprised Caucasian (self-declared) adult (> 16 years) patients who had enrolled in the Registry and volunteered a blood sample prior to January 1st, 2006. These cases were gender-matched at a 1:3 ratio with healthy controls otherwise randomly selected from the WTCCC subset of the 1958 British Birth Cohort. DNA samples issued to the WTCCC from the 1958 British Birth Cohort were restricted to Caucasians (http://www.wtccc.org.uk/info/overview.shtml).

Control Data

Encrypted genome-wide data (Illumina 1.2M chip; Essex, United Kingdom) on healthy controls from the WTCCC subset of the 1958 British Birth Cohort were secured online from the European Genome-phenome Archive (EGA, www.ebi.ac.uk/ega). Decryption and SNP-specific data extraction were using Bcrypt (http://bcrypt.sourceforge.net) and GTOOL v0.4.1 (Oxford, United Kingdom), respectively.

Laboratory Methods

Blood samples for cases were stored at -80°C and thawed in batches prior to DNA extraction. DNA was isolated from sample aliquots using a Qiagen QIAamp DNA Blood Mini Kit (Hilden, Germany). The quality and quantity of extracted DNA was assessed using a NanoDrop ND-1000 Spectrophotometer (Wilmington, Delaware).

Assays were obtained from Applied Biosystems (Foster City, California). Reactions were prepared using a TaqMan Fast Universal Master Mix (2x), SNP Genotyping Assay Mix (40x), DNase-free water, and genomic DNA (10 ng). PCR amplification was completed using an ABI Prism 7900HT Sequence Detection System under the following conditions: 10 minutes at 95°C followed by 45 cycles at 92°C for 15 seconds and at 60°C for 1 minute. Concordance for quality control samples was between 99% and 100% for all assays.

Clinical Data

H. pylori infection status, disease severity and chronicity, and responses to splenectomy and to isolated first courses of prednisolone, IVIg, anti-D, and rituximab were recorded for patients for whom data were available. A platelet count less than 10×10^9/L at presentation served as a surrogate marker for severe disease. Consensus guidelines by the International Working Group on ITP[53] were used to define chronicity (> 1 year) and complete response (> 100×10^9/L within specified intervals) to treatment.

Statistical Analyses

Associations of candidate SNPs with primary ITP were evaluated using logistic regression models of log-additive co-dominance. Results were reported as per rare allele odds ratios (ORs) with 95% confidence intervals (CIs). To avoid underpowered testing, a minimum of 23 cases, calculated using Quanto v1.2.4 (Los Angeles, California), was required for association analyses with clinical variables.[*]

[*] This threshold was formulated using the following specifications: design = unmatched case-control (1:2), inheritance: log-additive, rare allele frequency = 0.35, baseline risk = 0.20, and estimated OR = 3.0.

Ethics

The study was conducted under the auspices of the UK Adult ITP Registry, an active, linked-anonymised repository of hospital-based clinical data (demographics, bleeding events, ITP-specific treatments, laboratory results, and co-morbid conditions) and biological samples (whole blood, ~EDTA) of adult patients with primary ITP established to uncover information pertaining to disease pathogenesis, treatment effectiveness, and co-morbid burden. Launched in late-2007, the UK Adult ITP Registry has been approved for multi-centre operation by the London Research Ethics Committee (Reference: 07/H0718/57) until mid-2017 and is sponsored by Barts and The London NHS Trust. Informed consent was obtained from every patient prior to enrolment, and ethical approval secured for genome-wide SNP testing.

Results

Demographics & SNP-Primary ITP Association Results

A total of 206 (145 females, 61 males) Caucasian adults with primary ITP were sex-matched (1:3) with 618 (435 females, 183 males) healthy controls from the WTCCC subset of the 1958 British Birth Cohort. Observed genotype frequencies for the six candidate SNPs did not differ significantly from expected results under the assumption of HWE in healthy controls (Table 3-3). Per allele ORs and 95% CIs are depicted in Figure 3-1 and reveal a statistically significant association between *TNFA* -308 g>a and primary ITP (OR = 1.34 [95% CI, 1.03-1.75]; p = 0.03).

Clinical Data, SNP- H. pylori Infection & Severity Association Results

Clinical data were available for 93 (45.1%) patients. The median post-diagnosis follow-up time was 6.0 years (range: 0.4-34.7 years). Severe disease was observed in 27 of 76 (35.5%) patients for whom presenting counts were documented. Chronic and acute disease was observed in 77 (82.8%) and 12 (12.9%) patients, respectively (insufficient follow-up prevented classification of 4 [4.3%] patients). Twenty-three (43.4%) of 53 *H. pylori* tests were positive. Patients were treated in order of frequency with prednisolone, IVIg, splenectomy, anti-D, and rituximab. Of the clinical variables captured, only *H. pylori* infection and disease severity met the threshold established for proceeding with analyses, and no significant associations were observed (Figure 3-2).

Table 3-3: Observed Genotype Frequencies and SNP-Disease, *H. pylori* Infection, and Severity Association Results

Gene	SNP *rs Number*	Genotype	SNP Disease Association				SNP-*H. pylori* Infection Association				SNP-Severity Association			
			Controls (%)	Cases (%)	OR (95% CI)	P	Controls (%)	Cases (%)	OR (95% CI)	P	Controls (%)	Cases (%)	OR (95% CI)	P
IL10	-592 c>a *rs1800872*		HWE *p = 0.61*		1.14 (0.88-1.48)	0.33	HWE *p = 0.42*		0.97 (0.43-2.22)	0.95	HWE *p = 0.62*		1.40 (0.65-2.98)	0.39
		CC	382 (61.8)	119 (59.5)			17 (56.7)	13 (56.5)			33 (68.7)	17 (63.0)		
		CA	205 (33.2)	66 (33.0)			10 (33.3)	8 (34.8)			13 (27.1)	7 (25.9)		
		AA	31 (5.0)	15 (7.5)			3 (4.2)	2 (8.7)			2 (4.2)	3 (11.1)		
TNFA	-308 g>a *rs1800629*		HWE *p = 0.82*		1.34 (1.03-1.75)	0.03	HWE *p = 0.85*		1.62 (0.71-3.70)	0.26	HWE *p = 0.42*		1.01 (0.48-2.13)	0.99
		GG	403 (65.2)	115 (57.2)			15 (51.7)	9 (40.9)			25 (53.2)	12 (46.1)		
		GA	191 (30.9)	74 (36.8)			12 (41.4)	9 (40.9)			17 (36.2)	13 (50.0)		
		AA	24 (3.9)	12 (6.0)			2 (6.9)	4 (18.2)			5 (10.6)	1 (3.9)		
TGFB1	-509 c>t *rs1800469*		HWE *p = 0.91*		1.08 (0.85-1.38)	0.52	HWE *p = 0.85*		1.15 (0.48-2.74)	0.75	HWE *p = 0.58*		1.09 (0.52-2.27)	0.82
		CC	308 (49.9)	100 (49.0)			15 (51.7)	11 (47.8)			27 (56.3)	14 (51.9)		
		CT	255 (41.3)	81 (39.7)			12 (41.4)	10 (43.5)			17 (35.4)	11 (40.7)		
		TT	54 (8.8)	23 (11.3)			2 (6.9)	2 (8.7)			4 (8.3)	2 (7.4)		
IL1A	-889 c>t *rs1800587*		HWE *p = 0.88*		0.82 (0.63-1.07)	0.15	HWE *p = 0.14*		1.63 (0.64-4.18)	0.31	HWE *p = 0.58*		1.03 (0.47-2.26)	0.94
		CC	297 (48.1)	97 (51.9)			21 (72.4)	11 (52.4)			29 (61.7)	15 (62.5)		
		CT	264 (42.7)	80 (42.8)			6 (20.7)	9 (42.8)			15 (31.9)	7 (29.2)		
		TT	57 (9.2)	10 (5.4)			2 (6.9)	1 (4.8)			3 (6.4)	2 (8.3)		
IL10	-819 c>t *rs1800871*		HWE *p = 0.59*		1.18 (0.91-1.53)	0.21	HWE *p = 0.42*		1.03 (0.45-2.35)	0.95	HWE *p = 0.67*		1.34 (0.62-2.86)	0.46
		CC	382 (62.3)	94 (47.5)			17 (56.7)	12 (54.5)			31 (67.4)	17 (63.0)		
		CT	201 (32.8)	85 (42.9)			10 (33.3)	8 (36.4)			13 (28.3)	7 (25.9)		
		TT	30 (4.9)	19 (9.6)			3 (10.0)	2 (9.1)			2 (4.3)	3 (11.1)		
IL4R	q576r *rs1801275*		HWE *p = 0.99*		1.14 (0.88-1.48)	0.32	HWE *p = 0.52*		1.74 (0.72-4.17)	0.22	HWE *p = 0.27*		0.84 (0.42-1.67)	0.61
		AA	379 (61.3)	121 (60.8)			19 (63.3)	10 (43.5)			25 (52.1)	16 (59.3)		
		AG	210 (34.0)	60 (30.1)			9 (30.0)	11 (47.8)			17 (35.4)	8 (29.6)		
		GG	29 (4.7)	18 (9.1)			2 (6.7)	2 (8.7)			6 (12.5)	3 (11.1)		

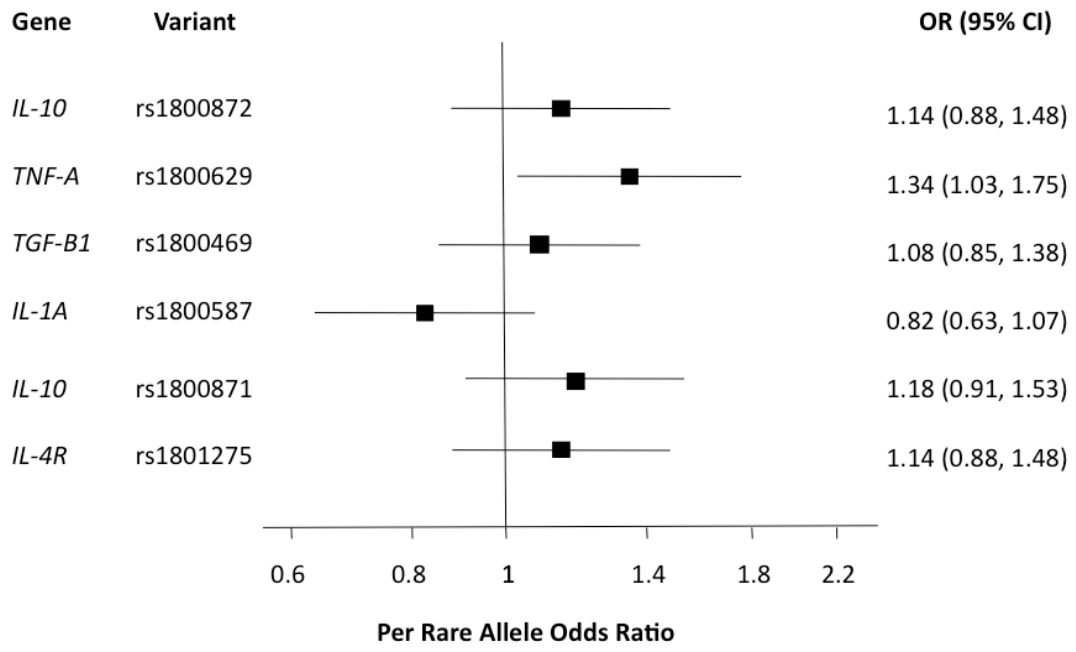

Figure 3-1: Results of logistic regression modelling of associations between 6 functional candidate SNPs and primary using a sample of 206 Caucasian adults with primary ITP gender-matched with 618 healthy Caucasian controls. A statistically significant association was observed between the disease and *TNF-A* -308 g>a.

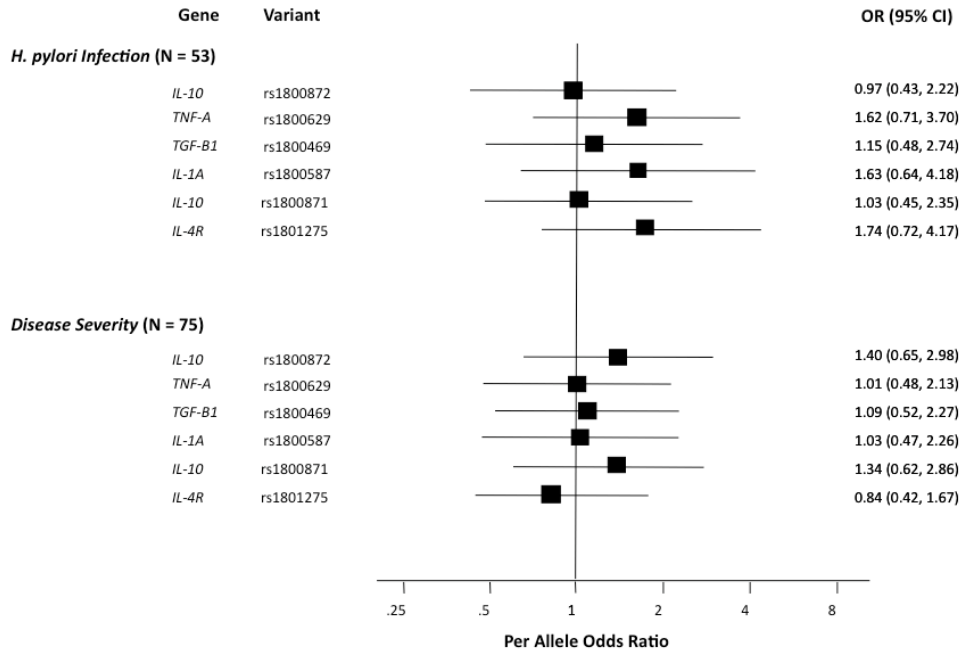

Figure 3-2: Results of logistic regression modelling of associations between 6 functional candidate SNPs and both disease severity and *H. pylori* infection in Caucasian adults with primary ITP. No statistically significant associations were observed.

Discussion

While the failure to observe significant associations between the candidate SNPs and either disease severity or *H. pylori* infection may reflect a true lack of association, it is similarly possible that the conducted analyses possessed inadequate statistical power, the primary limitation of the study. The minimum threshold of cases established for the above association analyses was predicated on the remote possibility of uncovering a pronounced effect (*e.g.*, OR = 3.0). Were significant SNP associations with these outcomes to exist, they would likely be less pronounced and require a greater sample size to detect. For example, assuming the study design detailed in Figure 3-3 and a rare allele frequency of 0.1, 267 cases, or patients with severe disease or *H. pylori* infections, would be required to uncover an OR of 1.6.

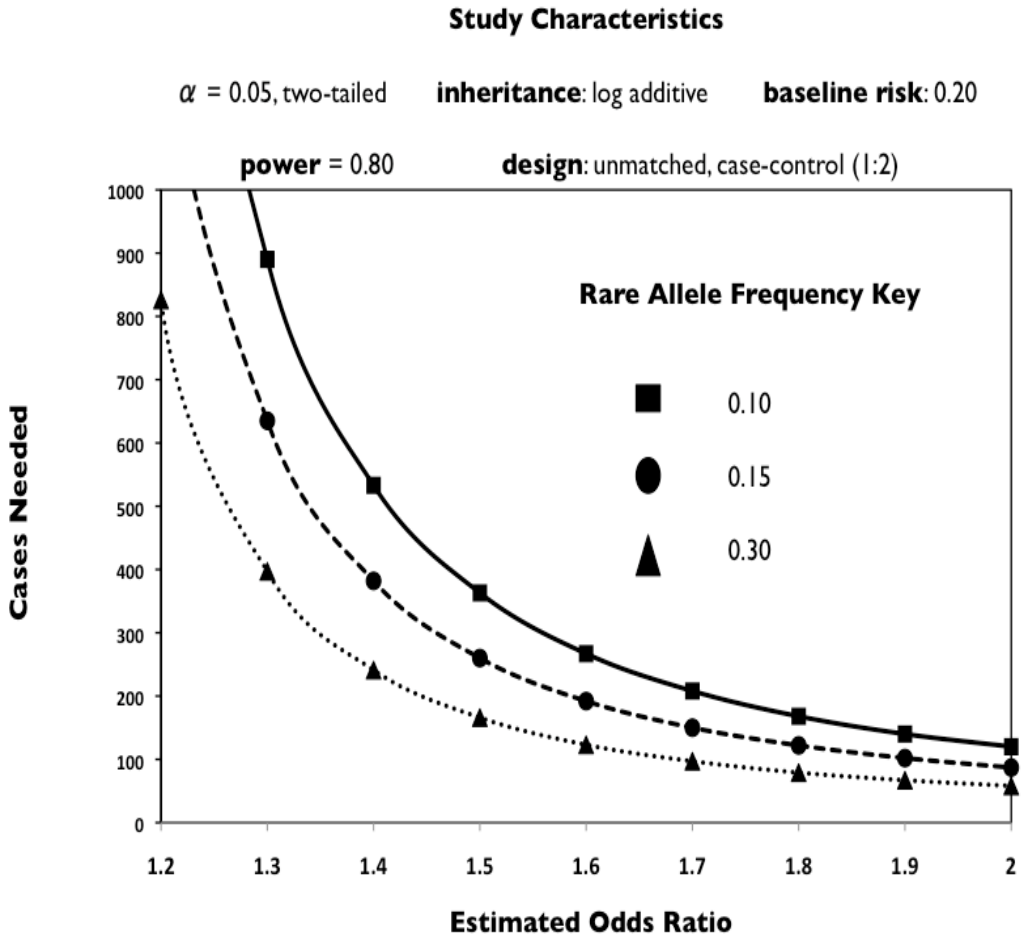

Figure 3-3: A graph of cases necessary to detect moderate SNP-severity, *H. pylori* infection, and treatment response associations.

Two further potential limitations of this study should be noted relating to methodological differences in the evaluation of cases and controls. First, the use of different genotyping platforms may have led to spurious differences in SNP frequencies between cases and controls. Second, reliance on self-declared ethnicity for cases may have resulted in a greater admixture, or accidental inclusion of non-Caucasian patients, than among controls, for whom principal component analyses had been performed. Although the possibility that these systematic differences may have biased the results of the study cannot be excluded, their impact would have likely been minimal. The candidate SNPs under investigation were all directly typed (*i.e.,* data imputation was not performed). Furthermore, as the rare allele at *TNFA -308* is more common among Caucasians than among individuals of Japanese, Chinese, or African ancestry, the accidental inclusion of non-Caucasian cases in our study would have likely biased our odds ratio toward, rather than away, from the null.

Coupled with these limitations, the principle finding of a borderline statistically significant OR of 1.34 (95% CI, 1.03-1.75; p = 0.03) raises the question as to whether the uncovered association between *TNFA* -308 g>a and primary ITP among Caucasian adults may be a false positive result. The high frequency of false positives among initially reported findings in genetic association studies has been well documented, with subsequent investigations often unable to replicate these results.[54] As Wacholder *et al.*[54] note, evaluation of the likelihood of false positives should include not only the reported p-value, which reveals the probability of a study yielding data as, or more, extreme than that observed given a true null hypothesis (*i.e.*, that no association exists), but also on the statistical power of the study, and the prior probability of a true association. While the p-value and statistical power of the study are admittedly moderate, the prior probability for this candidate SNP association was arguably high, prompting me to speculate that it may represent a true positive result.

The observed association between *TNFA* -308 g>a and primary ITP among Caucasian adults is consistent with the latter's categorisation as a Th1-disease. TNF-α plays a critical role in the development of pro-inflammatory Th1 responses,[55] and the rare allele at -308 has been linked with elevated production of the cytokine. In a Dutch study of patients with inflammatory bowel disease and healthy controls, Bouma *et al.*[37] reported that participants with TNF-E and TNF-C haplotypes secreted

the highest and lowest levels of TNF-α, respectively.[37] Crucially, these haplotypes only differed at the -308 g>a position (TNF-E: -308 a; TNF-C: -308 g). As a functional polymorphism, then, *TNFA* -308 g>a may serve as a marker of pro-inflammatory events.

Three previous studies in primary ITP have evaluated *TNFA* -308 g>a. In a pilot study involving 37 cases and 218 controls, Foster *et al.*[23] reported a significant association (association-p = 0.0032; HWE-p = 0.02)* between the SNP and chronic ITP in children.. Investigations by Satoh *et al.*[15] and Suzuki *et al.*[21] were conducted among Japanese cohorts. Suzuki *et al.*, however, evaluated the association of the SNP with *H. pylori* infection in patients with primary ITP and not primary ITP itself. Furthermore, although Satoh *et al.* did not observe a significant association, the frequency of the rare allele in the Japanese population is significantly lower than among Caucasians (HapMap-JPT: 2.3% vs. HapMap-CEU: 21.7%†). Hence, for a Japanese population, between 1,502 and 3,796 patients with primary ITP would be required to detect an association of similar magnitude as presented in this report.

In conclusion, the finding of a significant association between *TNFA* -308 g>a and primary ITP in Caucasian adults represents a promising step forward, highlighting the merits of further research into the genetic contribution to the Th1/Th2 imbalance in the disease. Importantly, this study represents the largest investigation of SNPs in primary ITP to date. Thus, its primary limitation of suboptimal power provides a convincing rationale for the development of an international registry for adults with primary ITP. Such a registry would enable validation of reported SNP-disease associations, as required of this study, while laying the necessary foundation for the long-term goal of genome-wide association studies (GWAS).

* Notably, the observed genotype frequencies among controls differed significantly from expected frequencies under an assumption of HWE in Foster *et al.*'s study. While such a deviation may have resulted from chance or bias (*e.g.*, genotyping errors), it is also possible for it to have been real (*e.g.*, selection). Nonetheless, results where HWE-deviations are observed must be interpreted cautiously.
† Allele frequency data obtained from the National Center for Biotechnology Information SNP database.

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Chapter 4: The utility of autologous ^{111}In-labelled platelet sequestration studies in patients with primary ITP prior to splenectomy

Summary

Although splenectomy is widely recognised as an effective therapy for primary ITP, the risk of peri- and post-operative complications, limited data on long-term relapse, and an observed lack of efficacy in roughly one-third of patients suggest merit in further research into possible pre-surgical predictors of response. The effectiveness of platelet sequestration site in predicting short, medium, and long-term responses to splenectomy was evaluated through multivariable logistic regression modelling of outcome data on patients who had undergone autologous ^{111}In-labelled platelet sequestration studies at Barts and The London NHS Trust. In total, 256 patients with primary ITP underwent scanning, with 91 (35.5%) proceeding to splenectomy. Logistic regression models revealed significantly increased adjusted (gender, age at splenectomy, and mean platelet lifespan) odds of response in purely or predominantly splenic versus mixed or hepatic, splenectomised patients at 1-3 (OR: 7.47 [95% CI, 1.89-29.43]) and 6-12 (OR: 4.85 [95% CI, 1.04-22.54]) months post-splenectomy and at last follow-up (median post-surgical time: 3.8 years [range: 0.5-13.1 years]; OR: 5.39 [95% CI, 1.34-21.65]). An independent, inverse association of age at splenectomy and long-term response (OR: 0.95 [95% CI, 0.91-0.99]) was further observed. Taken together, these results highlight splenectomy as better treatment for younger patients with purely or predominantly splenic sequestration.

Introduction

Although primary ITP specialists have increasingly adopted a less interventional management paradigm, therapy is still indicated for patients with symptomatic thrombocytopenia, at high risk of bleeding, or undergoing procedures likely to induce blood loss.[1] The spleen serves as a principal site of anti-platelet antibody production and platelet phagocytosis.[2,3] Splenectomy has, thus, proved a particularly effective treatment for primary ITP, with a complete response (CR)* observed in approximately two-thirds of adults.[4] However, the procedure carries a risk of peri- and post-operative complications, including intra-abdominal haemorrhages,[5] TEs,[6] and opportunistic post-splenectomy infections.[7] Although low,[8] the combined risk of these complications in primary ITP provides sufficient impetus to investigate potential pre-operative predictive variables of response, particularly when coupled with a known risk of non-CR in a third of patients and limited data on long-term relapse.

To date, a number of such predictors have been posited, including 1) response to corticosteroid and IVIg therapies, 2) platelet turnover and lifespan, 3) patient age, 4) duration of disease, 5) platelet-bound immunoglobulin, and 6) site of platelet destruction as determined by radionuclide labeling techniques**.** As noted in Chapter 1, Kojouri *et al.*[4] reported inconclusive evidence in support of this last variable in a systematic review of the effectiveness of splenectomy among adult patients with primary ITP.[4] Evaluating a series of 15 studies, the authors found the site of platelet destruction to be correlated with post-splenectomy outcome in 6,[9-14] uncorrelated in 8,[15-22] and inconclusive in 1.[23]

* A normal platelet count (> 150 x 10^9/L or as defined and at least > 100 x 10^9/L) and no ITP-specific treatment 30 days post-splenectomy.

Table 4-1(a): Past Investigations of Radioisotope-Labelled Platelet Sequestration Studies in Primary ITP (1976-1984)

Author, Year (Country) *Inclusion Criteria Beyond 1997 ASH Diagnosis Guidelines*	Study Size	Sex	Age at Surgery	Platelet Label *Origin*	Sequestration Classification	Outcome Success Criteria	Results
Ries, 1976 (USA) •*Included 28 patients with primary ITP and 6 patients with systemic lupus erythematosus (SLE) or other systemic autoimmune disease-associated thrombocytopenia*	Scans 34 Surgeries 34	Female 24 Male 10	Mean 26.2 years	^{51}Cr *Not stated*	Not stated	Post-Surgery Assessment (a) 1 month (b) Last follow-up Mean: 22.8 months Complete response (CR) Count > 150 x 10^9/L Partial response (PR) Count = 50–150 x 10^9/L	1 month post-surgery Splenic: 26 (76.5%) CR: 21 (80.8%); PR: 4 (15.4%) Mixed: 8 (23.5%) CR: 6 (75.0%); PR: 1 (12.5%) Last follow-up Splenic CR: 24 (92.3%); PR: 2 (7.7%) Mixed CR: 7 (87.5%); PR: 1 (12.5%)
Ikkala *et al.*, 1978 (Finland)	Scans 20 Surgeries 24	Total Cohort Female 34 Male 11	N ≤ 40 years 15 N > 40 years 9	^{51}Cr *Not stated*	Not stated	Post-Surgery Assessment Median: 60 months Range: 24–96 months CR Count > 100 x 10^9/L No bleeding PR Count = 50-100 x 10^9/L No major bleeding	Splenic: 9 (45.0%) CR: 8 (88.9%); PR: 0 Normal: 8 (40.0%) CR: 5 (62.5%); PR: 2 (25.0%) Hepatic: 3 (15.0%) CR: 1 (33.3%); PR: 1 (33.3%)
Burger *et al.*, 1978 (Hungary) •*Count < 50 x 10^9/L* •*Corticosteroids impractical or ineffective after 3 months*	Scans 86 Surgeries 40	Female 33 Male 7	Not stated	^{51}Cr *Not stated*	•Spleen:liver ratio (S:L) at 10% survival Splenic S:L > 1.5 Mixed S:L & L:S < 1.5 Hepatic L:S > 1.5	Post-Surgery Assessment Likely > 2 years CR Count > 100 x 10^9/L PR 40–100 x 10^9/L NR < 40 x 10^9/L Relapse Undefined	Splenic: 28 (70.0%) CR: 25 (89.3%); PR: 1 (3.6%) Mixed: 7 (17.5%) CR: 3 (4.3%); PR: 2 (2.9%) Hepatic: 5 (12.5%) CR: 2 (40.0%); PR: 0 Relapse: 4 (10.0%)
den Ottolander *et al.*, 1984 (Holland) •*Count < 60 x 10^9/L*	Scans 87 Surgeries 49	Not stated	Not stated	^{51}Cr *Not stated*	Not stated	Post-Surgery Assessment Not stated CR Count > 120 x 10^9/L; 0-1 Relapses PR Count = 60-120 x 10^9/L Relapse Count ≤ 60 x 10^9/L *after* Count > 120 x 10^9/L	Splenic: 42 (85.7%) CR: 28 (66.7%); PR: 5 (11.9%) Mixed or Hepatic: 7 (14.3%) CR or PR: 4 (57.1%)

Table 4-1(b): Past Investigations of Radioisotope-Labelled Platelet Sequestration Studies in Primary ITP (1987-1992)

Author, Year (Country) *Inclusion Criteria Beyond 1997 ASH Diagnosis Guidelines*	Study Size	Sex	Age at Surgery	Platelet Label *Origin*	Sequestration Classification	Outcome Success Criteria	Results
Russo *et al.*, 1987 (Italy) •*Chronic thrombocytopenia (count < 100 x 10^9/L)*	Scans 119 Surgeries 119	Female 78 Male 41	Mean ~29 years	^{51}Cr *If count > 50 x 10^9/L* *Autologous* *If count ≤ 50 x 10^9/L and if not transfused or pregnant* *Allogeneic*	•Spleen:precordium & liver:precordium ratios at 50% survival classified as follows, respectively Splenic > 2.9 & ≤ 1.5 Mixed > 2.9 & > 1.5 Hepatic ≤ 2.9 & > 1.5	Post-Surgery Assessment 1 month CR Count > 150 x 10^9/L PR Count = 80–149 x 10^9/L Relapse Count < 150 x 10^9/L at last follow-up (mean: 128 months) among CR patients at 1 month	Splenic: 82 (68.9%) CR: 66 (80.5%); PR: 10 (12.2%) Mixed: 21 (17.6%) CR: 14 (66.7%); PR: 2 (9.5%) Hepatic: 3 (2.5%) CR: 0; PR: 2 (66.7%) Relapse: 11 of 89 (12.4%) *NB: Results of 13 patients with diffuse pattern not shown*
Siegel *et al., 1989* (USA) •*Unresponsive to prednisolone or unacceptably high dose required*	Scans 59 Surgeries 21	Not stated	Median 40 years Range 20–84 years	^{111}In-oxine *Autologous*	•S:L ratio at 24 hours Splenic S:L > 1.2 Mixed 0.8 ≤ S:L ≤ 1.2 Mixed S:L < 0.8	Post-Surgery Assessment 1-1.5 months CR Count > 180 x 10^9/L No treatment	Splenic: 15 (71.4%) CR: 9 (60.0%) Mixed: 3 (14.3%) CR: 2 (66.7%) Hepatic: 3 (14.3%) CR: 2 (66.7%)
Lamy *et al.*, 1992 (France) •*Count < 50 × 10^9/L*	Scans 111 Surgeries 51	Scan Cohort Female 66 Male 45	Not stated	^{111}In-oxine *Autologous*	•Relative increase in spleen and liver activity from 0.5 hours to last day of activity Splenic S increase > 1.2 Mixed S & L increase > 1.2 Hepatic L increase > 1.2	Post-Surgery Assessment 3 months CR Count > 150 x 10^9/L PR Count = 50-150 x 10^9/L	Splenic: 38 (74.5%) CR: 33 (86.8%); PR: 1 (2.6%) Mixed: 9 (23.7%) CR: 2 (22.2%); PR: 0 Hepatic: 4 (10.5%) CR & PR: 0

Table 4-1(c): Past Investigations of Radioisotope-Labelled Platelet Sequestration Studies in Primary ITP (1993-1997)

Author, Year (Country) *Inclusion Criteria Beyond 1997 ASH Diagnosis Guidelines*	Study Size	Sex	Age at Surgery	Platelet Label *Origin*	Sequestration Classification	Outcome Success Criteria	Results
Naouri *et al.*, 1993 (France) •*Chronic thrombocytopenia*	Scans 35 Surgeries 72	Female 58 Male 14	Mean 36.4 years	^{51}Cr *Allogeneic*	Not stated	Post-Surgery Assessment (a) 3 months (b) Last follow-up Median 5.4 years Range: 0.6–11.9 years CR Count > 120 x 10^9/L PR Count = 50-120 x 10^9/L	Splenic: 22 (62.9%) CR & PR: Not stated Hepatic: 2 (5.7%) CR & PR: Not stated •Splenic pattern a predictor of CR at 3 months (p = 0.02) but not at last follow-up. *NB: Results of patients with mixed and diffuse patternsnot shown*
Winde *et al.*, 1996 (Germany) •*Count < 100 x 10^9/L*	Scans 68 Surgeries 72	Female 42 Male 30	Mean 48.0 years	^{51}Cr *Not stated*	Not stated	Post-Surgery Assessment As below for > 2 months or at 2 weeks post-surgery CR Count > 150 x 10^9/L No bleeding PR Count = 50-100 x 10^9/L No bleeding	•CR & PR results by sequestration site not stated *"Patients with clearly defined splenic thrombocytolysis had a …more than 90 percent [chance] of experiencing [CR], whereas patients with hepatic thrombocytolysis more frequently had an unsuccessful…follow-up"*
Najean *et al.*, 1997 (France) •*Extension of Najean et al., 1991* •*Chronic thrombocytopenia Count < 100 × 10^9/L* •*Mean platelet lifespan < 48 hours* •*No pregnant women & young children*	Scans 580 Surgeries 268	Not stated	Not stated	^{111}In-oxine *Autologous*	•Relative increase in S:L from 0.5 hours to 20% survival Purely Splenic S:L increase > 2 Predominantly Splenic 1.4 < S:L increase ≤ 2 Mixed 0.8 < S:L increase ≤ 1.4 Hepatic 0.8 < S:L increase	Post-Surgery Assessment Undefined CR Count > 300 × 10^9/L PR Count > 100 × 10^9/L	Purely splenic: 138 (51.5%) CR: 132 (95.7%); PR: 4 (2.9%) Predominantly splenic: 74 (27.6%) CR: 66 (89.2%); PR: 7 (9.6%) Mixed: 29 (10.8%) CR: 11 (37.9%); PR: 7 (24.1%) Hepatic: 16 (6.0%) CR: 2 (12.5%); PR: 1 (6.3%)
Louwes *et al.*, 1999 (Holland) •*Count < 100 × 10^9/L* •*Mean platelet lifespan ≤ 9.2 days*	Scans 141 Surgeries 53	Scan Cohort Female 91 Male 50	Not stated	^{111}In-tropolone *Autologous*	Not stated	Post-Surgery Assessment Undefined CR Count > 150 x 10^9/L No prednisolone PR Count = 50-150 x 10^9/L No prednisolone	Splenic: 32 (60.4%) CR or PR: 28 (87.5%) Hepatic: 8 (15.1%) CR or PR: 2 (25.0%) Non-splenic: 13 (24.5%) CR or PR: 9 (69.2%)

Table 4-1(d): Past Investigations of Radioisotope-Labelled Platelet Sequestration Studies in Primary ITP (2000-Present)

Author, Year (Country) *Inclusion Criteria Beyond 1997 ASH Diagnosis Guidelines*	Study Size	Sex	Age at Surgery	Platelet Label *Origin*	Sequestration Classification	Outcome Success Criteria	Results
Radaelli *et al.*, 2000 (Italy) •*Unresponsive to prednisolone*	Scan 59 Surgeries 65	Female 45 Male 20	Mean 37.3 years	Pre-1988 ^{51}Cr *Not stated* Post-1988 ^{111}In-oxine *Not stated*	Not stated	Post-Surgery Assessment Mean: 129.8 months CR Count > 100 x 10^9/L PR Count = 50-100 x 10^9/L	Splenic: 25 (42.4%) CR or PR: 21 (84%) Mixed or Hepatic : 6 (10.2%) CR or PR: 3 (50%) Other: 28 (47.4%) CR or PR: 21 (75%)
Rossi *et al.*, 2002 (Italy) •*Unresponsive to prednisolone: Count ≤ 50 × 10^9/L after 1 month* •*Decision to undergo splenectomy partially based on sequestration pattern*	Scans 71 Surgeries 25	Not stated	Mean 43.4 years	^{111}In-oxine *Autologous*	•Imaging at 15, 30, 60, 120 and 240 minutes & 1, 2, 3, and 4 days post-injection •Maximum increase in splenic and hepatic uptake expressed as a percentage of baseline values: ≤ 30% (0), 30–49% (1), 50-99% (2), ≥ 100% (3)	Post-Surgery Assessment (a) Not stated (b) Last follow-up Median 3 months Range: 1-8 months CR Count > 100 x 10^9/L PR Count > 50 x 10^9/L Relapse Count < 50 x 10^9/L After initial CR or PR	Initial CR CR: 23 (92.0%), PR: 2 (8.0%) Relapse 6 (24.0%) Non-relapsed vs. relapsed No difference in splenic (1.7 vs. 1.3 [p = 0.45]) and hepatic (0.5 vs. 0.6 [p = 0.88]) uptake
Bourgeois *et al.*, 2002 (France) •*Extension of Fenaux et al., 1989* •*Chronic thrombocytopenia* •*Lifespan ≤ 5 days*	Scans 255 Surgeries 183	Female 119 Male 64	Median 32 years Range 4–75 years	^{111}In-oxine *Autologous*	•Relative increase in the S:L ratio from 0.5 hours to MPLS Splenic S:L increase > 1 Hepatic S:L increase < 1	Post-Surgery Assessment 3 months Response (R) Count > 100 x 10^9/L Relapse Relapse: Success at least 1 year post-surgery among patients with success at 3 months	Splenic: 157 (86.7%) R: 131 (83.4%), relapse: 7 (4.5%) Hepatic: 24 (13.3%) R: 10 (41.7%), Relapse: 2 (20.0%) Long-Term Difference noted in mean S:L ratio between responders (2.21) and non-responders (1.37), p = 0.025 *NB: 2 patients who underwent splenectomy added in follow-up study*

The methodologies of these studies were, however, notably heterogeneous with regard to the isotopic label used, sequestration classification scheme, patient inclusion criteria, and both the definition of and period for response (Table 4-1). Moreover, 6[15,18,19,21-23] (66.7%) uncorrelated or inconclusive studies assessed fewer than 50 patients undergoing both a platelet sequestration study and a splenectomy. The power of many of these investigations to detect an association between sequestration site and response to splenectomy was therefore limited. These shortcomings underscore the need for an additional, suitably powered study, which incorporates currently standardised isotopic labelling techniques and consensus criteria for response evaluation.

Barts and The London NHS Trust is the principal ITP referral centre for adults in the UK, and autologous ^{111}In-labelled platelet sequestration studies have been routinely practiced there as part of the planning process prior to splenectomy for over a decade. Using the cohort of patients with primary ITP who have undergone such studies, the objectives of our investigation were as follows: 1) to evaluate the prognostic utility of platelet sequestration site as a predictor of short, medium, and long-term response to splenectomy through multivariable logistic regression modelling and 2) to compare long-term outcomes from splenectomy with alternate therapeutic approaches in patients with primary ITP.

Methods

Study Design and Population

A retrospect cohort design was adopted. Inclusion in the study cohort was restricted to patients with primary ITP as defined by 2003 BCSH guidelines[24] who had undergone a ^{111}In-labelled autologous platelet sequestration study at Barts and The London NHS Trust between March 1st, 1994 and December 31st, 2008. All patients were required to have platelet counts between 5 x 10^9/L and 100 x 10^9/L at the time of their scan, and no antibody therapies were permitted four weeks prior to this date.

Autologous ^{111}In-Labelled Platelet Sequestration Study Protocol

Autologous ^{111}In platelet-labelling was performed in accordance with recommendations of The International Committee for Standardization in Hematology Panel on Diagnostic Applications of Radionuclides.[25] Briefly, whole blood (50 mL) was withdrawn from the patient using acid citrate glucose (ACG, 6:1) and centrifuged at 90–100 *g* for 10 minutes. The supernatant (platelet rich plasma [PRP]) was adjusted to a pH of 6.5 by further addition of ACG and centrifuged at 650 *g* for 10 minutes. The resulting supernatant (platelet poor plasma [PPP]) was used (0.3–0.5 mL) to suspend the derived platelet pellet. Tropolone (50 µL, 0.054% [w/v] tropolone in HEPES-saline buffer [pH 7.6]) was then added to the suspension, followed immediately by ^{111}In-chloride (4 MBq). The mixture was incubated at room temperature for 5 minutes, washed with PPP (5 mL), and centrifuged at 650*g* for 10 minutes. The supernatant (containing unbound ^{111}In-tropolone) was discarded, leaving ^{111}In-tropolone-labelled platelets, which were suspended in PPP (5 mL) and injected into a peripheral vein.

At 0.5, 3, 24, and 48 hours post-injection, blood samples (5 mL) were taken and centrifuged in a two-stage process to yield PPP and a platelet pellet. Surface counting was performed on both products, with a gamma camera set to register ^{111}In-photon peaks at 171 and 245 KeV. Counting, corrected for background and decay, was similarly performed over the heart, liver, and spleen at these intervals using the geometric mean of anterior and posterior views. Regions of interest were drawn over these organs using the 0.5-hour acquisition and transferred to subsequent acquisitions to ensure analysis of identical anatomical areas.

A semi-logarithmic graph of platelet-associated radioactivity versus time was constructed to derive the platelet half-clearance time ($t_{50\%}$), which in turn was used to compute the mean platelet lifespan (MPLS) using the algorithm, $1.44\times t_{50\%}$.[17,26] Najean *et al.*'s proposed classification system[26] was adopted to catergorise platelet sequestration. The relative increase in the spleen:liver (S:L) ratio from 0.5 hours to the time of 80% platelet destruction was calculated under this scheme, with an increase of greater than 2.0 characterised as purely splenic sequestration, between 1.4 and 2.0 as predominantly splenic sequestration, between 0.8 and 1.4 as mixed sequestration, and less than 0.8 as hepatic sequestration.

Follow-Up

At least one-year following the sequestration study, haematologists were questioned as to whether their patients had undergone a splenectomy. For patients who had, 1-3 and 6-12 months post-surgery platelet counts were requested in addition to treatment status and platelet count at last follow-up. Haematologists of patients who did not have a splenectomy were alternatively asked to supply a reason (open-field) why surgery was not performed in addition to providing the treatment status and platelet count of their patients at last follow-up (Figure 2-3). Official death certificates were obtained for patients who died over the course of follow-up.

Outcomes

The outcome of interest among the splenectomised population was CR, defined using recent guidelines by the International Working Group on ITP[27] as a platelet count greater than 100 x 10^9/L at 1-3 (short-term) and 6-12 (medium-term) months post-surgery and a platelet count less than 100 x 10^9/L or reliance on an ITP-specific treatment at last follow-up (long-term). For the descriptive comparison of long-term outcomes among splenectomised and non-splenectomised patients, platelet count and ITP-specific treatment status at last follow-up were assessed independently.

Exposures & Covariates

Platelet sequestration site, dichotomised as purely or predominantly splenic and mixed or hepatic, comprised the principal exposure in the study. Additional covariates included gender, age at surgery (continuous), and MPLS (continuous).

To create a suitable comparative cohort for splenectomised patients, a categorical variable of reason(s) for not undergoing surgery was constructed. Open-field responses from haematologists were grouped into one of the following ten categories: 1) an asymptomatic or only mildly symptomatic patient; 2) co-morbid condition(s); 3) a spontaneous, first-line (corticosteroids or IVIg) treatment-based, or unspecified remission; 4) a well-controlled patient on a first-line treatment; 5) physician, parent, or patient choice 6) a non-first-line treatment-based remission; 7) a well-controlled patient on a non-first-line treatment; 8) platelet sequestration study test result; 9) multiple reasons; 10) no reason provided. Patients listed as having undergone surgery for reason 5, 6, 7, 8, or 9 (without their reasons classifiable under

1, 2, 3, or 4 above) were selected to comprise the comparative, non-splenectomised cohort.

Statistical Analyses

The investigation was framed with an aim to determine whether autologous ^{111}In-labelled platelet sequestration studies could identify patients who would benefit from surgery. Unadjusted and adjusted (gender, age at splenectomy, and MPLS) odds ratios (ORs) and 95% confidence intervals (95% CIs) for CR at 1-3 and 6-12 months post-surgery and last follow-up were modelled using logistic regression (STATA 9.2; College Station, Texas).

For all secondary analyses, differences between groups were evaluated at α = 0.05 using Student's t (two-tailed), Kruskal-Wallis, and Pearson's chi-square tests for normally distributed, non-parametric, and dichotomous outcomes, respectively. Determination of normality for continuous variables was based on visual inspection of both box-plots and histograms.

Date of primary ITP diagnosis was available for a subset of patients undergoing autologous ^{111}In-labelled platelet sequestration studies. A pre-hoc decision was therefore made to perform subgroup analyses, incorporating disease duration at the time of splenectomy as an additional covariate in the multivariable logistic regression models outline above.

To supplement these analyses, histograms were constructed of platelet counts among the purely or predominantly splenic and mixed or hepatic, splenectomised cohorts at 1-3 months and 6-12 months post-surgery. Similar histograms were made of platelet counts at last follow-up for both these cohorts and the comparative, non-splenectomised cohort.

Ethics

The study was conducted under the auspices of the UK Adult ITP Registry, an active, linked-anonymised repository of hospital-based clinical data and biological samples of adult patients with primary ITP established to uncover information pertaining to disease pathogenesis, treatment effectiveness, and co-morbid burden. Launched in late-2007, the UK Adult ITP Registry has been approved for multi-centre operation by the London Research Ethics Committee (Reference: 07/H0718/57) until mid-2017 and is sponsored by Barts and The London NHS Trust. Informed consent was obtained from every patient prior to enrolment. All patients who were children (< 16 years) at the time of their autologous ^{111}In-labelled platelet sequestration study had reached adulthood by the time of study initiation.

Results

Autologous ^{111}In Sequestration Studies

A total of 272 autologous ^{111}In sequestration studies were conducted at Barts and The London NHS Trust between March 1st, 1994 and December 31st, 2008. As illustrated by Figure 4-1, scans were performed on 256 patients with primary ITP, 9 with secondary ITP (7 with SLE-induced thrombocytopenia and 2 with HIV-induced thrombocytopenia), 4 with congenital or familial thrombocytopenia, and 3 with Evans syndrome. The female-to-male ratio of patients with primary ITP was 1.7:1, and the median age at the time of scan was 38.0 years (range: 7.3-75.4 years).

Platelet sequestration patterns among the primary ITP cohort were heterogeneous: 68 (26.6%) patients exhibited purely splenic sequestration, 76 (29.7%) predominantly splenic sequestration, 60 (23.4%) mixed sequestration, and 52 (20.3%) hepatic sequestration (Figure 4-2). The median MPLS in patients with primary ITP was 28.2 hours (range: 6.8-407.0 hours). A significant difference ($p = 0.491$) was not noted between the MPLS of patients with purely or predominantly splenic platelet sequestration and patients with mixed or hepatic platelet sequestration (median: 25.9 hours [range: 6.8-407.0 hours] vs. 31.1 hours [range: 7.2-271.0 hours]).

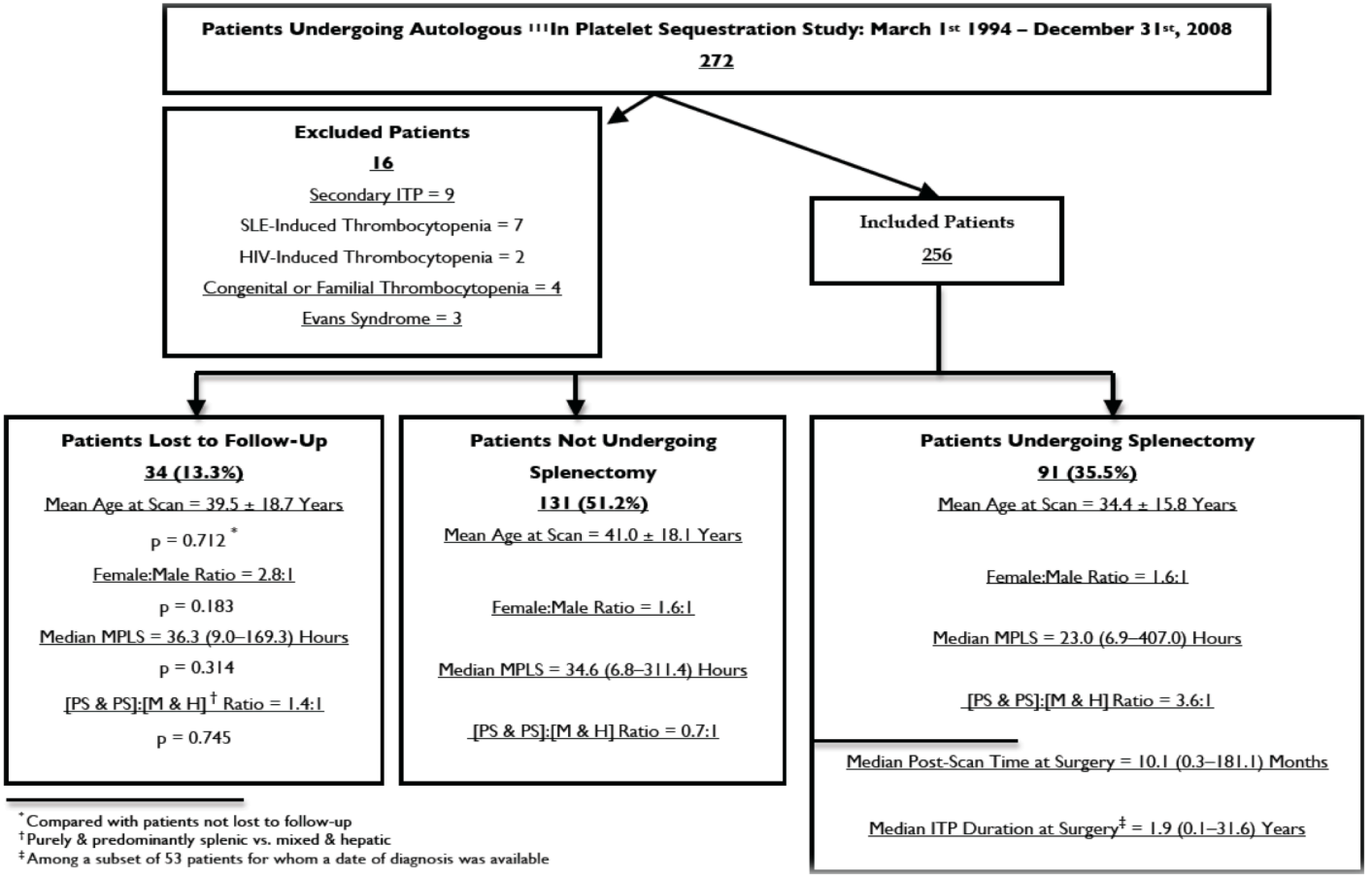


Figure 4-1: An overview of the study population. In total, 265 patients with primary ITP underwent autologous ^{111}In-labelled platelet sequestration studies at Barts and The London NHS Trust between March 1994 and December 2008, with 91 (35.5%) of patients subsequently proceeding to splenectomy.

Follow-Up

Over one-third of patients with primary ITP (N = 91 [35.5%]) elected to undergo a splenectomy following their autologous ^{111}In sequestration study while roughly half (N = 131 [51.2%]) did not. Thirty-four patients (13.3%) were lost to follow-up (Figure 4-1). These patients did not differ significantly from patients not lost to follow-up with regard to age at the time of the scan (mean: 39.5 ± 18.7 years vs. 38.3 ± 17.5 years; p = 0.712), sex (female:male ratio: 2.8:1 vs. 1.6:1; p = 0.183), platelet sequestration pattern (purely and predominantly splenic: mixed & hepatic ratio: 1.4:1 vs. 1.3:1; p = 0.745), or MPLS (median: 36.3 hours [range: 9.0-169.3 hours] vs. 27.6 hours [range: 6.8-407.0 hours]; p = 0.314).

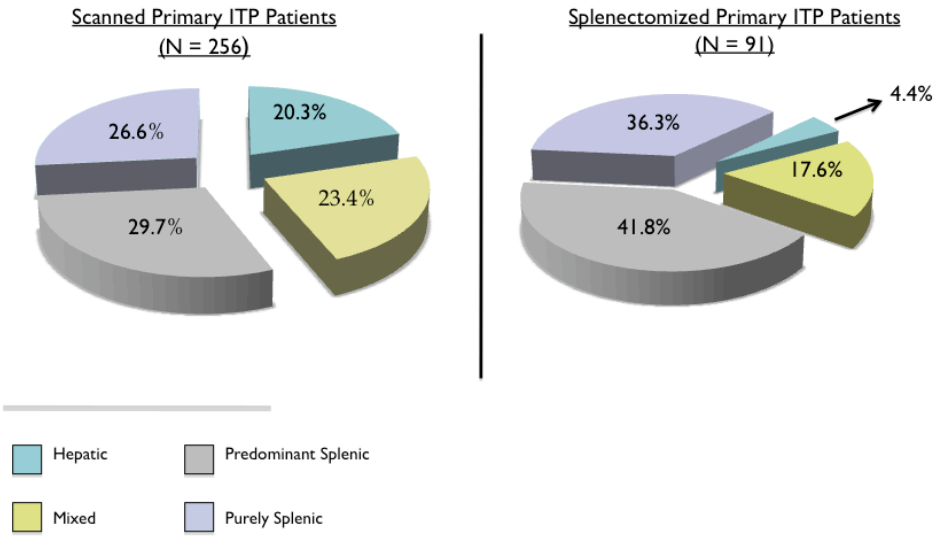


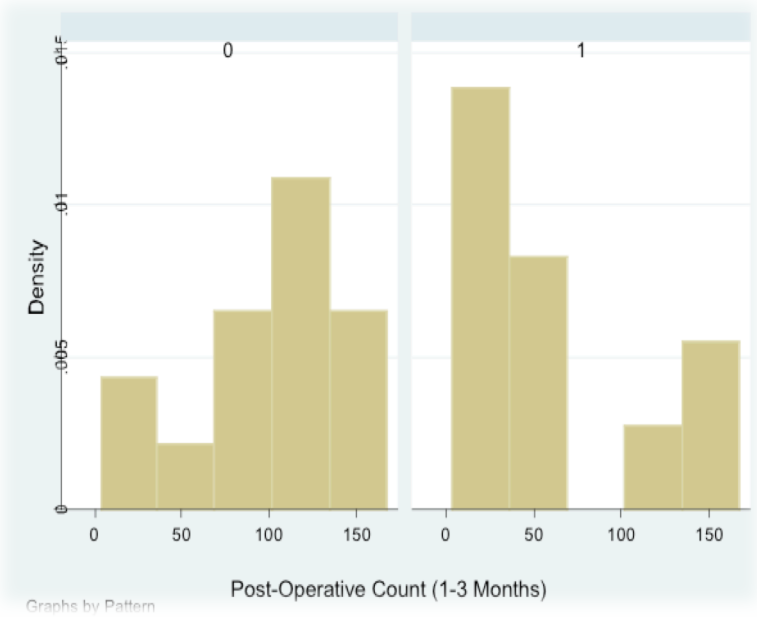
Figure 4-2: Sequestration patterns of patients who underwent an autologous ^{111}In-labelled platelet study and patients who subsequently underwent splenectomy. A statistically significant difference (p < 0.001) was observed between the proportions of purely or predominantly splenic versus mixed or hepatic patterned patients proceeding to splenectomy.

Haematologist supplied reasons for patients not undergoing splenectomy, documented in Table 4-2, most commonly included 1) the platelet sequestration test result (*i.e.,* a mixed or hepatic sequestration finding suggesting a lower probability of response to splenectomy, N = 39 [29.8%]); 2) an asymptomatic or only mildly symptomatic patient (N = 26 [19.8%]); and 3) patient, parent, or clinician choice (N = 23 [16.0%]).

Of 71 patients with purely or predominantly splenic sequestration who opted for a splenectomy, 57 (80.3%) had evaluable results at 1-3 months post-surgery, 54 (76.1%) at 6-12 months post-surgery, and 69 (97.2%) at last follow-up. CRs were observed in 50 (87.7%), 44 (87.0%), and 60 (87.0%) of these patients, respectively. Of 20 patients undergoing splenectomy with a mixed or hepatic pattern of platelet sequestration, 19 (95.0%) had evaluable results at 1-3 months post-surgery, 18 (90.0%) at 6-12 months post-surgery, and 20 (100%) at last follow-up, with 7 (36.8%), 5 (27.8%), and 7 (35.0%) experiencing CRs, respectively. Median post-surgical times at last follow-up were 3.8 years (range: 0.5-13.1 years) for the purely or predominantly splenic, splenectomised cohort, over which no deaths were reported, and 2.8 years (range: 0.5-10.8 years) for the mixed or hepatic, splencetomised cohort, over which the following 2 non-haemorrhagic fatalities were observed: 1) acute myocardial infarction and coronary atherosclerosis, 2) cerebral infarction and type II diabetes. Histograms of patient platelet counts within these cohorts at the aforementioned assessment intervals are illustrated in Figures 4-3 and 4-4.

Table 4-2: Reasons for Not Proceeding to Splenectomy

Reason (s)	Number (%)
Test Result	39 (29.8)
Asymptomatic or Only Mildly Symptomatic	26 (19.8)
Patient, Parent, or Clinician Choice	23 (16.0)
Treatment-Based Remission	11 (8.4)
Corticosteroids	5
Rituximab	2
IVIg	1
Various Treatments	1
Platelet Transfusion & Corticosteroids	1
Unspecified Treatment	1
Multiple Reasons	8 (6.1)
Test Result & Patient Choice	5
Test Result & Mildly Symptomatic Patient	1
Test Result & Treatment-Based Remission (Corticosteroids)	1
Co-Morbid Condition & Only Mildly Symptomatic Patient	1
Co-Morbid Condition(s)	5 (3.8)
Spontaneous Remission	5 (3.8)
Unspecified Remission	4 (3.1)
Well Maintained on Non-First Line Treatment	3 (2.3)
Azathioprine	1
Rituximab	1
Tranexamic Acid	1
No Reason Provided	6 (4.6)
Total	**131**



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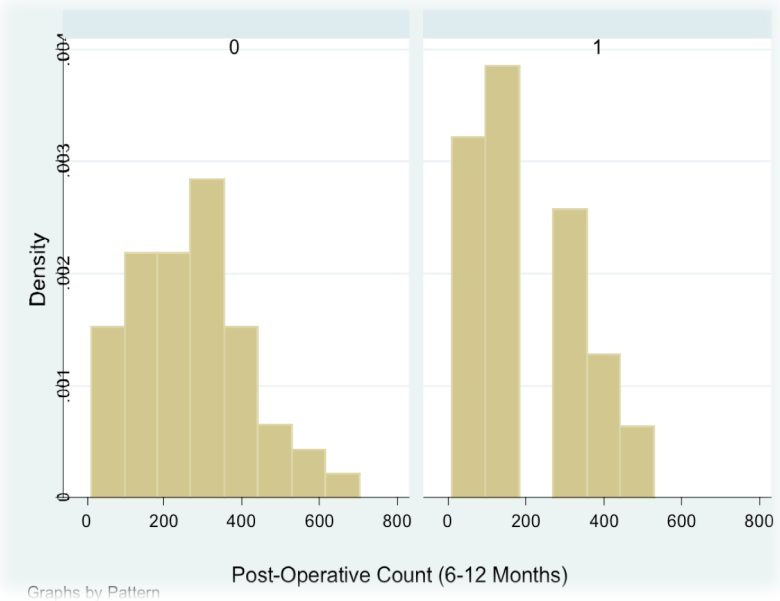

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Figure 4-3: Histograms of platelet counts (×10⁹/L) among the purely or predominantly splenic (0) and mixed or hepatic (1), splenectomised cohorts at 1-3 months (a) and 6-12 (b) months post-splenectomy. The size of each bar is representative of the proportion of patients in the cohort with a platelet count in the specified range.

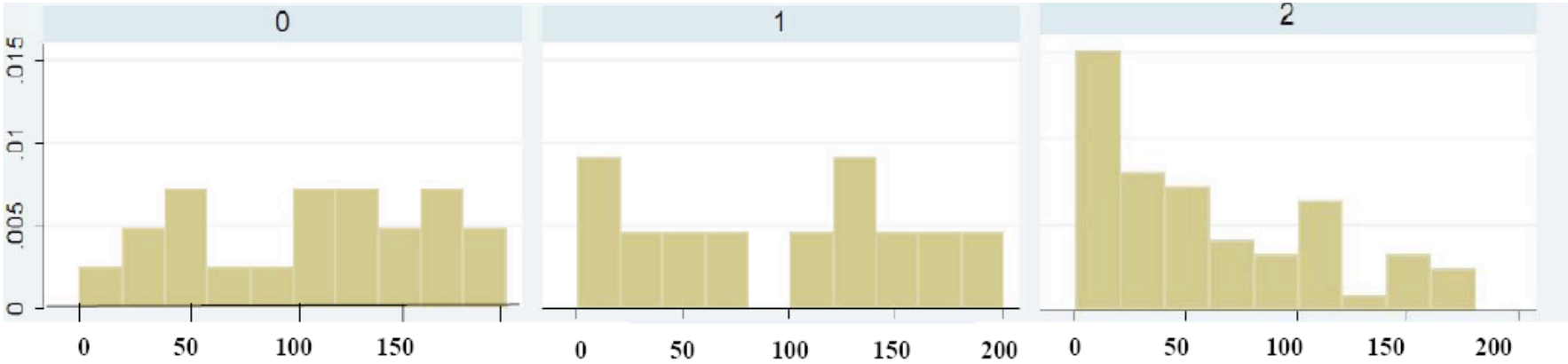

Figure 4-4: Histograms of platelet counts (×10⁹/L) among the purely or predominantly splenic (0) and mixed or hepatic (1), splenectomised cohorts and the comparative, non-splenectomised cohort (2) last follow-up. The size of each bar is representative of the proportion of patients in the cohort with a platelet count in the specified range.

Multivariable Modelling

Unadjusted and adjusted (gender, MPLS, and age at splenectomy) odds of short, medium, and long-term CR to splenectomy were increased in patients with purely or predominantly splenic versus mixed or hepatic sequestration. Logistic regression modelling yielded statistically significant, unadjusted ORs at 1-3 months post splenectomy (OR = 4.96 [95% CI, 1.41-17.46]) and last follow-up (OR = 3.59 [95% CI, 1.13-11.40]), but not at 6–12 months post-splenectomy (OR = 2.58 [95% CI, 0.70-9.49]). Adjusted ORs at these intervals were all significant: 1-3 months post-splenectomy (OR = 7.47 [95% CI, 1.89-29.43], 6-12 months post-splenectomy (OR = 4.85 [95% CI, 1.04-22.54], and last follow-up (OR = 5.39 [95% CI, 1.34-21.65]. The median post-surgery time at last outpatient visit was 3.8 years (range: 0.5-13.1 years). No other potential, pre-surgical, predictive variable achieved statistical significance at 1-3 months and 6-12 months post-surgery (Table 4-3). However, age at the time of surgery did emerge as an independent predictor of CR at last follow-up (OR: 0.95 [95% CI, 0.91-0.99]).

Table 4-3: Multivariable Logistic Regression Results

Post-Surgery Assessment Time	N	Pattern PS/PS vs. M/H* OR (95% CI)†	Gender Female vs. Male OR (95% CI)	MPLS OR (95% CI) *Per Hour*	Age at Surgery OR (95% CI) *Per Year*	Post-ITP Duration OR (95% CI) *Per Month*
Primary Analyses						
1-3 Months	71	7.47 (1.89-29.43)	0.76 (0.18-3.13)	1.01 (0.98-1.03)	0.99 (0.94-1.03)	
6-12 Months	67	4.85 (1.04-22.54)	3.69 (0.85-15.91)	1.01 (0.98-1.05)	0.96 (0.91-1.00)	
Last Follow-Up Median: 3.8 years (Range: 0.5-13.1 years)	84	5.39 (1.34-21.65)	2.70 (0.75-9.68)	1.01 (0.98-1.04)	0.95 (0.91-0.99)	
Subgroup Analyses						
1-3 Months	43	8.01 (1.63-39.50)	1.45 (0.26-8.07)	1.00 (0.96-1.05)	1.00 (0.95-1.06)	1.10 (0.90-1.35)
6-12 Months	39	23.40 (1.87-292.33)	0.21 (0.02-1.78)	1.04 (0.97-1.10)	1.00 (0.93-1.08)	0.93 (0.74-1.17)
Last Follow-Up Median: 4.1 years (Range: 0.5-13.1 years)	52	9.20 (1.54-54.94)	0.20 (0.04-1.11)	1.03 (0.98-1.08)	0.96 (0.91-1.02)	1.32 (0.77-2.27)

Inclusion of the lapse of time between primary ITP onset and splenectomy as an additional covariate for patients with available diagnosis dates, while altering the OR point estimates, did not influence the statistical significance of platelet sequestration pattern as a pre-surgical predictor of response at 1-3 months post splenectomy (OR: 8.01 [95% CI, 1.63-39.50], 6-12 months post-splenectomy (OR: 23.40 [95% CI, 1.87-292.33]), or last follow-up (OR: 9.20 [95% CI, 1.54-54.94]).

Splenectomised vs. Comparative, Non-Splenectomised Cohorts

In total, 72 (55.0%) patients with primary ITP who did not undergo splenectomy met inclusion criteria for the comparative, non-splenectomised cohort. CRs were observed in 13 (19.1%) of these patients over a median post-scan follow-up time of 2.7 years (range: 0.1-11.4 years; Table 4-4). Over this period, the following two non-haemorrhagic deaths were reported: 1) parieto occiptal ependymoma and 2) chronic myelomonocytic leukaemia and renal impairment/septicaemia. While the median platelet count of this cohort (58 × 10^9/L [range: 2-358 × 10^9/L]) was significantly lower than both the mixed or hepatic, splenectomised cohort (199 × 10^9/L [range: 2-577 × 10^9/L]) and the purely or predominantly splenic, splenectomised cohort (292 × 10^9/L [range: 14-589 × 10^9/L]; $p < 0.001$) at last follow-up (Figure 4-4), the proportion of patients on treatment within the comparative, non-splenectomised cohort was comparable to that of the mixed or hepatic, splenectomised cohort (28 [41.2%] patients vs. 6 [30.0%] patients, respectively; $p = 0.47$). Furthermore, of the 28 patients in the comparative, non-splenectomised cohort on treatment at last follow-up, 8 (28.6%) were solely taking low-dose corticosteroids (< 10 mg/day). Notably, only 3 (4.3%) splenectomised patients with purely or predominantly splenic platelet sequestration were on treatment at last follow-up (Table 4-4).

Table 4-4: Long-Term Outcomes of Study Participants

Cohort	Total N	Evaluable N	Complete Response N (%)	Post-Scan Time Median (Range) *Years*	Platelet Count Median (Range) *× 10⁹/L*	On Treatment N (%)
Purely or Predominantly Splenic, Splenectomised	71	69	60 (87.0)	3.0 (0.7-14.4)	292 (14-589)	3 (4.3)
Mixed or Hepatic, Splenectomised	20	20	7 (35.0)	3.0 (0.8-12.0)	199 (2-577)	6 (30.0)
Comparative, Non-Splenectomised	72	72-Count 68-Treatment	55 (80.9)	2.8 (0.1-11.4)	56 (1-358)	28 (41.2)

Discussion

The results of this investigation revealed increased odds of short, medium, and long-term response to splenectomy in patients with purely or predominantly splenic sequestration as determined by autologous ^{111}In-labelled platelet studies. CRs at these intervals were observed between 85% and 90% of splenectomised patients comprising this cohort, with reliance on treatment limited to 3 (4.3%) patients at last follow-up, a median post-surgery time of 3.8 years (range: 0.5-13.1 years). Patient age at the time of splenectomy additionally emerged as an independent predictor of long-term response, implicating a 5% increased odds of CR per year difference between patients, a finding consistent with a majority of past investigations.[4] Taken together, these results highlight splenectomy as an extremely effective treatment for younger patients with purely or predominantly splenic sequestration.

The observation of a significant association between platelet sequestration pattern and CR was not affected by the incorporation of primary ITP duration as an additional covariate in subgroup analyses (Table 4-3). Curiously, this association also did not appear tied to differences in median MPLS, estimates of which were similar between patients with purely or predominantly splenic (31.1 hours [range: 7.2-271.0 hours] and mixed or hepatic (25.9 hours [range: 6.8-407.0 hours]; p = 0.491) sequestration. These data suggest that the rate of platelet destruction may be independent of both the primary site of platelet destruction and the likelihood of response to splenectomy in patients with primary ITP, a different conclusion than reached by Siegel *et al.* [22]

Long-term outcomes, as measured by platelet count and ITP-specific treatment status, among patients indicated but not opting for splenectomy (*i.e.*, the comparative, non-splenectomised cohort) were poor in relation to splenectomised

patients with purely or predominantly splenic platelet sequestration. However, the treatment status of patients within this cohort was comparable to that of splenectomised patients with mixed or hepatic sequestration. At last follow-up, 28 (41.2%) and 6 (30.0%) patients from these cohorts were on treatment, respectively (Table 4-4). Moreover, when excluding patients taking solely low-dose corticosteroids, the difference between these proportions narrowed as follows: 20 (30.0%) vs. 5 (25.0%) patients, respectively.

More controversially, then, the study data may further support the adoption of a cautious approach to splenectomy for patients with mixed or hepatic sequestration. Coupled with increased recognition of the physiological importance of the spleen, potential long-term vascular complications following splenectomy for haematological disorders,[28] and arguably similar long-term clinical outcomes (*e.g.*, reliance on treatment) in patients indicated but not opting for splenectomy, the association platelet sequestration pattern with CR raises the question as to whether patients with mixed or hepatic platelet sequestration may fare comparably or better without splenectomy. The median platelet count of the comparative, control cohort (56×10^9/L [range: 1-358×10^9/L]) was admittedly lower than that of the mixed or hepatic, splenectomised cohort (199×10^9/L [range: 2-577×10^9/L]; $p < 0.001$). However, most patients, 83.8% (57 of 68 patients), in the former cohort had counts above what ITP specialists conventionally consider a primary threshold for increased risk of major bleeding events, 10×10^9/L (Figure 4-4).[29] Furthermore, no fatal haemorrhages were observed in this group over a median of 2.7 years (range: 0.1-11.4 years).

Four methodological strengths make our study an important addition to existing literature on the utility of platelet sequestration studies in patients with primary ITP. First, the use of ^{111}In-tropolone may have contributed to more accurate test results than previous investigations utilising either ^{51}Cr or ^{111}In-oxine. ^{111}In-tropolone has been reported by some research groups to exhibit better platelet labelling efficiency and imaging properties than ^{51}Cr.[30] It is additionally more soluble than ^{111}In-oxine in aqueous media, sparing possible platelet impairment from the addition of ethyl alcohol used with the latter radionuclide label.[31]

Second, the labelling of autologous platelets enabled accurate adjustment for MPLS in our logistic regression models, preventing potentially spurious findings that may have resulted from alloimunization.[32] Importantly, it was this substitution of

allogeneic platelets with autologous platelets by Ballem *et al*. that illustrated the existence of suboptimal levels of platelet production among a segment of primary ITP population.[33]

Implementation of separate, multivariable logistic regression models to gauge short, medium, and long-term post-splenectomy, thirdly, enabled adjustment for potential confounders such as age at the time of surgery.

Fourth, the creation of a cohort of patients indicated but not opting for splenectomy represented a novel construct by which to compare long-term outcomes in splenectomised and non-splenectomised patients. Although past investigations have aptly documented a high success rate for splenectomy in patients with primary ITP, it remained unclear whether equivalent outcomes were possible under an alternate management paradigm.

Four limitations to the study should similarly be noted. First, while data were collected on 91 patients undergoing splenectomy following an autologous ^{111}In-labelled platelet sequestration study, the results of the scan were not blinded to patients or their haematologists. As a result, only 20 (20.4%) patients with mixed or hepatic sequestration elected to undergo surgery. The significant difference in the proportions of patients with mixed or hepatic versus purely or predominantly splenic sequestration proceeding to splenectomy ($p < 0.001$; Figure 4-2) raises the possibility of selection bias, namely that mixed or hepatic patterned patients who opted for splenectomy were worse off than those who did not. Though plausible, it is similarly possible for haematologists to have encouraged surgery among mixed or hepatic patterned patients deemed more likely to succeed. To address this issue, post-hoc analyses were conducted on a subset of patients for whom pre-surgery platelet counts and post-diagnosis duration were available. Neither variable differed significantly ($p = 0.59$ and $p = 0.83$, respectively). Furthermore, mixed or hepatic patterned patients who underwent splenectomy were on average eleven years ($p = 0.01$) younger than those who did not, suggesting that results would have been biased toward, rather than away from, the null.

Second, though advocated by the International Working Group on ITP,[27] use of 100×10^9/L as a platelet count threshold for CR may have discounted clinically relevant responses to splenectomy. Ideally, effectiveness would have been assessed by therapeutic impact on not only platelet count but also the bleeding events and HRQoL. However, sufficient data were not available to gauge the effect of

splenectomy on the latter two variables, an inherent limitation of retrospective investigations. Despite this shortcoming, the relative distributions of platelet counts among the purely or predominantly and mixed or hepatic, splenectomised cohorts at each of the assessment intervals (Figures 4-3 and 4-4) suggest that a greater proportion of patients within the latter cohort continued to have platelet counts placing them at increased risk of major haemorrhage (*i.e.*, $< 10 \times 10^9$/L) following surgery.

Third, the algorithm used to capture MPLS yielded skewed results for patients with near-normal platelet lifespans, as it has been shown to perform poorly in situations of uniform destruction (*i.e.*, normal MPLS).[34] These errant results at the normal end of the MPLS spectrum may have been compounded by the limited time points at which blood samples were taken (0.5, 3, 24, and 48 hours post-injection), which may have hindered accurate regression of platelet associated radioactivity on time. However, as the vast majority of study patients, 94.5% (225 of 238 patients for whom MPLS was evaluable), exhibited a markedly reduced MPLS of less than 4 days, it is unlikely that the multivariable models were appreciably biased.

Fourth, haematologists' supplied reasons for patients not undergoing splenectomy were subjective and susceptible to possible recall bias. Use of these reasons nevertheless likely resulted in an improved picture of probable long-term outcomes had patients in the splenectomised cohort not opted for surgery than evaluation of results from the non-splenectomised cohort as a whole.

In conclusion, while this investigation demonstrates utility in including autologous ^{111}In-labelled platelet sequestration studies as an adjunct predictive tool prior to splenectomy in primary ITP, the aforementioned limitations illustrate that considerable work is still needed on this topic. Potentially useful next steps include a meta-analysis of observational studies and the development of an international prospective cohort study. The latter project would permit critical appraisal of proposed refinements to the scanning protocol and the incorporation data from autologous ^{111}In-labelled platelet sequestration studies, which were beyond the scope of this investigation (*e.g.*, platelet production levels).

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Chapter 5: Effects of eradication of *Helicobacter pylori* infection in patients with primary ITP

Summary

Multiple single-centre studies have been conducted on the effect of eradication therapy on platelet counts in *Helicobacter pylori* (*H. pylori*)-infected patients with primary ITP. To synthesise and interpret the results of these investigations, a meta-analysis was performed. PubMed, EMBASE and Cochrane databases were interrogated for English-language articles detailing studies of 15 or more patients. Twenty-five such studies were identified, encompassing 696 evaluable patients. To enable comparisons of response to eradication therapy, recently adopted criteria by the International Working Group on ITP were employed to re-catergorise individual study results. Owing to observed heterogeneity, DerSimonian and Laird random-effects models were used to pool data across studies, revealing a mean complete response (> 100 × 10^9/L) of 42.7% (95% CI, 31.8%-53.9%) and a response (> 30 × 10^9/L and at least double the baseline count) of 50.3% (95% CI, 41.6%-59.0%). Linear regression demonstrated a significant, direct correlation. ($r = 0.351$, $p = 0.018$) between the reported response and the prevalence of infection in the source population. Given the limited invasiveness of diagnostic tests and both the limited toxicity and low costs of eradication therapy, these findings support the routine testing for and eradication of *H. pylori* infection in patients with primary ITP in regions with a high prevalence of infection.

Introduction

H. pylori is a Gram-negative microaerophilic bacterium capable of colonising the human stomach.[1] Evidence for its causal role in the onset of peptic ulcer disease was first uncovered by Marshall and Weiner[2] almost 30 years ago, spurring a dramatic increase in research into the bacterium and its possible involvement in the pathogenesis of other diseases. The prevalence of *H. pylori* infection is strongly correlated with the underlying socioeconomic conditions of a population, with higher rates of infection commonly observed in developing countries (Figure 5-1).[1] Conventional treatment for the eradication of the bacterium consists of triple therapy, a combination of amoxicillin, clarithromycin, and a proton pump inhibitor administered for one to two weeks.[1]

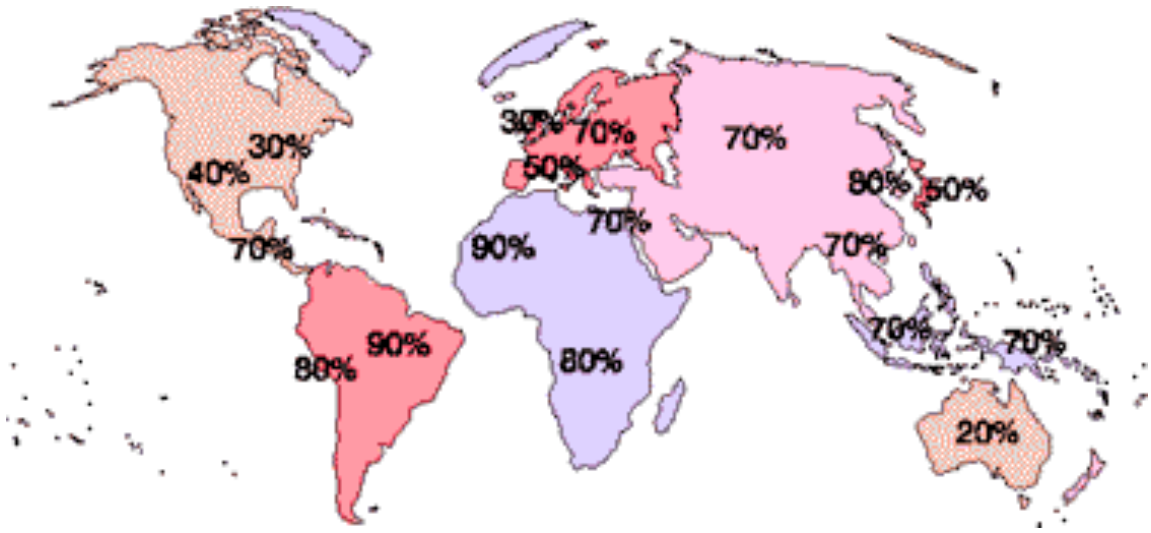

Figure 5-1: A map of the global prevalence (in percentages) of *H. pylori* infection. With the exception of Japan, higher rates of infection are typically present in developing countries. *Image courtesy of the Helicobacter Foundation.*

As discussed in Chapter 1, a relationship between *H. pylori* infection and primary ITP was first described by Gassbarini *et al.*[3] 1998, who reported a significant increase in platelet counts among 8 (72.7%) patients in whom the bacterium had been eradicated. Since then, numerous single-centre investigations have been conducted, yielding seemingly discrepant results. To synthesise and interpret these data, a meta-analysis of studies describing the effects of H. *pylori* eradication on platelet counts in adult patients with primary ITP was performed.

Methods

Literature Search

Searches for English language articles were conducted independently by two investigators in April 2008 using the electronic databases PubMed, EMBASE, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews, and the Cochrane Database of Abstracts of Reviews of Effects. PubMed was interrogated by combining the medical subject heading (MeSH) term "*Helicobacter pylori"* with "*thrombocytopenia"* or "*purpura, thrombocytopenic, idiopathic"*. A similar search strategy was employed for EMBASE whereas the Cochrane databases were reviewed for titles containing terms "*Helicobacter pylori"* or "*thrombocytopenia"*. The bibliographies of articles retrieved in this manner were further scanned for relevant publications. The abstracts of all articles secured from this combined search were subsequently evaluated on the criteria detailed below.

Criteria for Article Selection

An initial screening was performed for RCTs, cohort studies, and case-control studies evaluating the effects of *H. pylori* eradication on platelet counts in adult patients with primary ITP. Studies published in abstract form or involving less than 15 patients alone were deemed ineligible for inclusion, while those of 15 or more patients were reviewed to determine whether diagnoses had been made according to ASH guidelines.[4] Studies lacking reference to these guidelines were considered eligible only if secondary causes of thrombocytopenia had been clearly excluded (*e.g.*, HCV, HIV, and SLE).

Documentation of *H. pylori* infection was required to have been carried out by tests indicating active infection (*e.g.*, a ^{13}C-Urea Breath Test [UBT], a stool antigen test, or a histology of gastric biopsies). Serological tests were not considered adequate in this regard due to their comparatively low sensitivity and specificity.[5]

Finally, studies had to be evaluable for the effects of *H. pylori* eradication on platelet count over time. Disagreements over the final selection of articles were resolved by consensus in all cases. When a study had generated multiple publications, the latest or most informative version was used. Duplicate publications were used to provide information on baseline characteristics or methodology where necessary.

Data Extraction

Data extraction of each article was similarly carried out independently by two investigators. Individual-level patient data from studies were sought in the first instance. If patient-level data were not reported, group-level data were used. For the purposes of this review, the following data were collected: 1) study design and use of controls, 2) patient demographics, 3) previous and concomitant treatment for primary ITP, 4) baseline platelet count, 5) duration of primary ITP before eradication treatment, 6) diagnostic methods for *H. pylori* detection, 7) type and duration of the eradication regimen administered, 8) proportion of *H. pylori* infections eradicated, 9) criteria for platelet count response, 10) proportion of patients with platelet count responses, 11) time to platelet count responses, 12) follow-up and duration of platelet count responses, 13) effects of *H. pylori* eradication therapy on uninfected patients, and 14) predictors of platelet response. Discrepancies in extracted data were resolved by the two reviewers through joint reassessment of the original publication. For publications with missing or incomplete information, attempts were made to obtain additional data from study authors.

Assessment of Validity

The quality of cohort studies was assessed with the Newcastle-Ottawa Scale,[6] which assigns a score to three aspect of a study: selection bias, comparability of cohorts on the basis of design or analysis, and outcome assessment. An overall measure of quality was generated by adding these scores (highest score: 9).

The quality of RCTs were assessed with a scale created by Jadad *et al.*[7] In this instrument, 0 to 2 points are assigned for randomisation, 0 to 2 points are assigned for double blinding, and 0 to 1 points are assigned for a description of withdrawals and dropouts, resulting in a possible cumulative score of 0 to 5 (highest score: 5).

Assessment of Publication Bias

Susceptibility of the systematic review to publication bias, the tendency of studies with positive findings to be preferentially published, was formally assessed with Eggar's test, an inverse-variance weighted linear regression of the effect against its standard error, and was coupled with an informal visual inspection of funnel plot asymmetry.[8] Heterogeneity among studies was assessed with Cochran's Q test[9] and the I^2 test of inconsistency.[10]

Quantitative Data Synthesis

To enable comparisons of response to eradication therapy across studies, recently adopted criteria by the International Working Group on ITP[11] were employed to re-catergorise individual study results. Accordingly, a complete response (CR) was defined as a platelet count greater than 100 x 10^9/L and an overall response as a platelet count greater than 30 x 10^9/L and at least doubling of the baseline count. Where possible, mean platelet counts at baseline and at the time of response assessment were calculated, and sensitivity analyses performed in patients with baseline platelet counts greater than or less than 30 x 10^9/L.

As heterogeneity in response was anticipated, DerSimonian and Laird random-effects models were used to pool data for summary estimates.[12] Meta-analyses represent quantitative methods to combine data across similarly structured studies (*i.e.*, studies with comparable populations, designs, and outcome measures), resulting in greater analytic power. Statistical methods implemented in such analyses conventionally place less weight on results from smaller studies owing to their greater susceptibility to chance deviation. While fixed-effects meta-analysis models are based on an assumption of the existence of a single true value for a population parameter, random-effects models are premised on the existence of multiple normally distributed values of this parameter and, therefore, incorporate an additional measure of between-study variation. This component results in a relatively greater weight for smaller studies.

To stabilize variance, eradication and treatment-response proportions were subjected to a Freeman-Tukey arcsin square root transformation and back-transformed following quantitative data synthesis.[13] Proportions of 0 and 1 were adjusted as follows to enable their inclusion in study weighting: 0 = 1/(4 × sample size); 1 = (number of successes - 0.25)/(sample size). Results are presented as the weighted means with 95% CI.

Linear regression models were used for correlation analyses. Unweighted, chance-corrected κ values were calculated to assess agreement on study selection between the reviewers.[14] Assessment of publication bias and tests of heterogeneity were performed with Stata 8.2 (StataCorp, College Station, Texas); all other statistical tests were carried out with NCSS 2007 (NCSS, Kaysville, Utah).

Results

Selection of Studies

Figure 5-2 illustrates the results of our literature search. In total, 116 abstracts were reviewed. Thirty-five articles were selected for full-text evaluation. Of 25 non-English language publications excluded, 14 were noted to be narrative reviews, 8 were case reports, and one was a survey of current treatment strategies in Japan; only 2 articles detailed cohort studies (one with 11 primary ITP patients, the other with 20).

The proportion of agreement for initial study inclusion was 93% ($\kappa = 0.84$). Five duplicate publications and 4 studies reporting less than 15 patients were excluded as was one cohort study, which assessed *H. pylori* infections by serological tests alone. In total, 25 studies (1,555 patients with primary ITP) were included in our efficacy analyses.

Study Design

The designs of included studies are detailed in Table 5-1. Briefly, 22 prospective[3,16-36] and 2 restrospective[37,38] cohort studies were secured along with a single RCT.[15] Control populations were used in only 2 cohort studies.[27,28] In the study by Stasi *et al.*,[28] eradication therapy was only administered to *H. pylori*-positive patients with symptoms of dyspepsia or with platelet counts below 50×10^9/L.

Newcastle–Ottawa scores for cohort studies included in the meta-analysis were all greater than 5. The Jadad score for the sole RCT was 2; although the authors reported that patients were randomly assigned by concealed allocation, no information was provided on the precise method of randomisation.

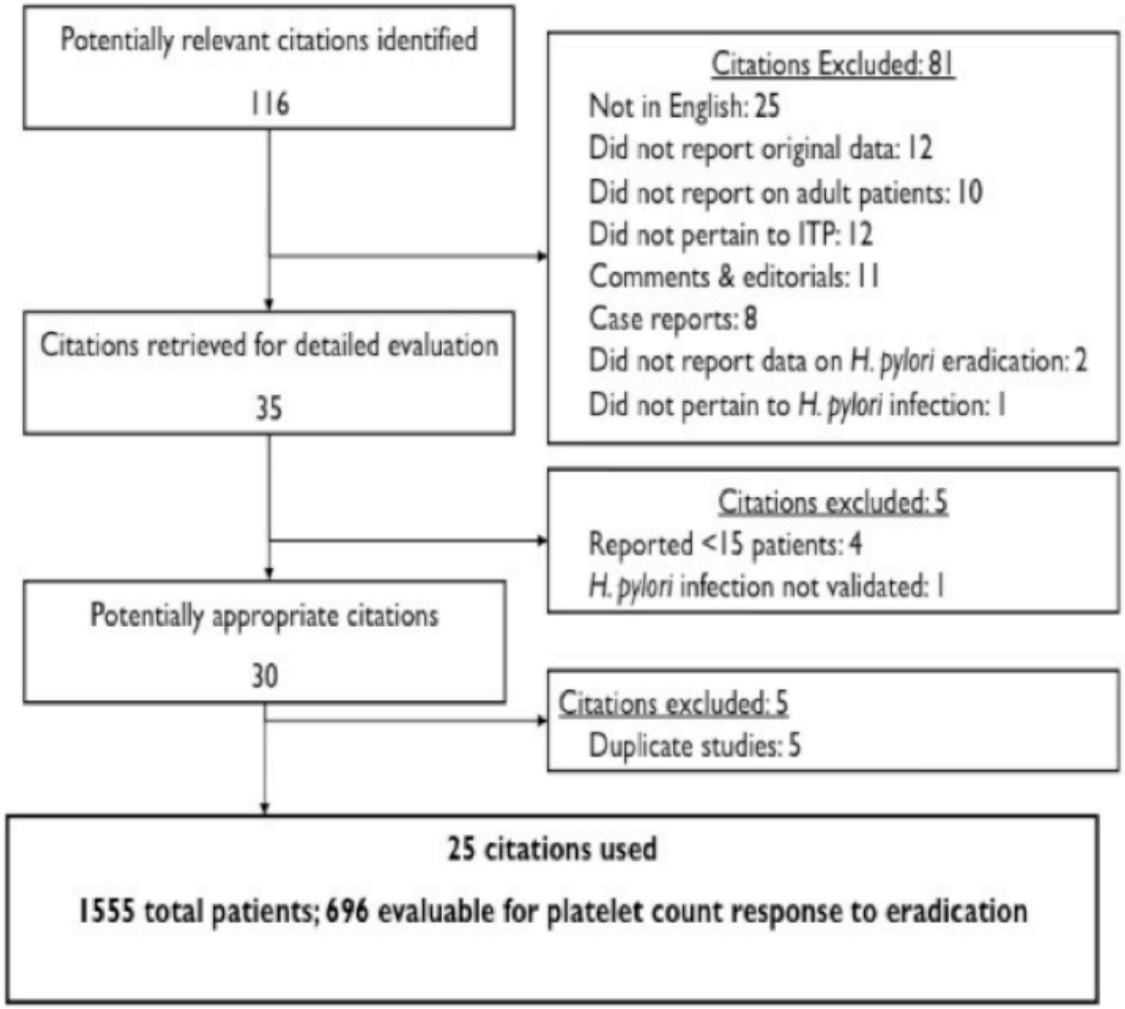

Figure 5-2: A step-wise overview of the literature search. Twenty-five studies were included in the systematic review.

Patient Demographics

Table 5-1 details the baseline characteristics of patients (N = 1,555) from studies included (N = 25) in the meta-analysis. The pooled prevalence of *H. pylori* infection among the study population was 65.0% (95% CI, 62.8%-67.3%) and did not differ significantly between males (63.4%) and females (66.5%). Information on baseline age was available for 1,132 patients from 13 studies.[3,17-22,28,29,31,33,36,37] *H. pylori*-positive patients were on average 10 years older than *H. pylori*-negative patients (57.5 years [95% CI, 56.4-58.5 years] vs. 45.3 years [95% CI, 43.7-46.8 years], respectively; $p < 0.001$). Infection status was not found to be significantly associated with gender, primary ITP duration, platelet count, or number of prior treatments.

Of 584 *H. pylori*-positive patients in 11 studies with a detailed treatment history,[17-22,28,30,35-37] 227 (38.9%) had not received any therapy prior to eradication while 341 (58.4%) had been administered corticosteroids. Conventional ITP treatments such as corticosteroids, azathioprine, and danazol were given concomitantly to eradication therapy in 121 (20.7%) patients.

Table 5-1: Baseline Characteristics of Patients in Included Studies

Authors (Year)	Country	Design	Total N	Gender *M/F**	Infected N (%)	Infected Age *Years*	Non-Infected Age *Years*	Infected Count *×10⁹/L*	Non-Infected Count *×10⁹/L*	ITP Duration *Months*	Concurrent Treatment
Gasbarrini *et al.* (1998)	Italy	Prospective Cohort	18	5/13	11 *(61)*	43 ± 14	49 ± 12	95 ± 39	103 ± 24	NR	NR
Jarque *et al.* (2001)	Spain	Prospective Cohort	56	18/38	40 *(71)*	54 (17-80)†		57 ± 22	58 ± 23	32 (2-50)	0/56
Kohda *et al.* (2002)	Japan	Prospective Cohort	40	12/28	25 *(62)*	54 ± 14	48 ± 13	67 ± 54	60 ± 41	41 ± 38	19/40
Hino *et al.* (2003)	Japan	Prospective Cohort	30	8/22	21 *(70)*	55 ± 15	51 ± 17	38 ± 20	22 ± 12	NR	7/30
Hashino *et al.* (2003)	Japan	Prospective Cohort	22	9/13	14 *(64)*	53 ± 13	42 ± 19	61 ± 26	63 ± 20	110 ± 81	8/22
Ando *et al.* (2003)	Japan	Prospective Cohort	61	12/49	50 *(82)*	58 ± 11	40 ± 16	56 ± 24	42 ± 24	78 ± 65	NR
Michel *et al.* (2004)	USA	Prospective Cohort	74	21/53	16 *(22)*	53± 16	39 ± 18	32 ± 15	26 ± 17[c]	122.4	9/25
Takahashi *et al.* (2004)	Japan	Prospective Cohort	20	5/15	15 *(75)*	54 ± 13	46 ± 18	40 ± 27	39 ± 22	51 ± 15	NR
Sato *et al.* (2004)	Japan	Prospective Cohort	53	16/37	39 *(74)*	62 (37-87)	52 (39-77	54 ± 17	59 ± 22	59 (6-624)	27/53
Ando *et al.* (2004)	Japan	Prospective Cohort	20	5/15	17 *(85)*	62 (38-83)		48 (4-86)	41 (12-82)	NR	NR
Nomura *et al.* (2004)	Japan	Prospective Cohort	42	15/27	28 *(66)*	NR	NR	29 ± 6	31 ± 5	NR	NR
Veneri *et al.* (2005)	Italy	Prospective Cohort	52	23/29	34 *(65)*	57 (24-72)	NR	57 ± 23	NR	NR	NR
Inaba *et al.* (2005)	Japan	Prospective Cohort	35	11/24	25 *(71)*	57 (25-82)		52 ± 26		40	0/35
Stasi *et al.* (2005)	Italy/UK	Prospective Cohort	137	57/80	64 *(47)*	58 ± 13	42 ± 16	42 ± 25	46 ± 23	25 ± 19	16/137
Fujimura *et al.* (2005)	Japan	Retrospective Cohort	435	120/315	300 *(69)*	59 ± 14	47 ± 16	NE	NE	98 ± 82	NR
Suzuki *et al.* (2005)	Japan	RCT‡	36	NR	25 *(69)*	NR	NR	55 ± 27	NR	NR	NR
Suvajdzic *et al.* (2006)	Serbia	Prospective Cohort	54	12/42	39 *(72)*	54 ± 13	42 ± 16	68 ± 32	78 ± 32	72 (12-360)	0/54
Ahn *et al.* (2006)	USA	Prospective Cohort	15	5/10	15 *(100)*	57± 19	NE	72 ± 45	NE	104 ± 79	11/15
Sayan et al. (2006)	Turkey	Prospective Cohort	34	22/12	20 *(59)*	51 ± 16	54 ± 18	37 ± 16	39 ± 16	19 ± 15	NR
Asahi et al. (2006)	Japan	Prospective Cohort	37	14/23	26 *(70)*	NR	NR	NR	NR	NR	NR
Kodama et al. (2007)	Japan	Prospective Cohort	116	32/74	67 *(58)*	58 ± 14	48 ± 17	39 ± 29	30 ± 24	NA	NA
Campuzano-Maya (2007)	Colombia	Retrospective Cohort	32	7/25	29 *(91)*	NR	NR	57 ± 38	NR	NR	NR
Estrada-Gomez (2007)	Mexico	Prospective Cohort	23	NR	14 *(61)*	NR	NR	NR	NR	NR	NR
Satake (2007)	Japan	Prospective Cohort	38	9/29	12 *(68)*	NR	NR	NR	NR	NR	NR
Emilia et al. (2007)	Italy	Prospective Cohort	75	36/39	38 *(51)*	58 ± 19	50 ± 21	41 ± 24	28 ± 18	24 ± 30	24/75

* Males/Females
† Median (range)
‡ Randomised-controlled trial

Publication Bias and Eradication of H. pylori infection

Treatment for *H. pylori* eradication consisted of triple-therapy in all studies save that by Estrada-Gomez *et al.*,[34] in which a four-drug regimen was used. Random-effects modeling revealed successful eradication in 89.7% (95% CI, 85.1%-93.5%) of treated patients. With the exception of the study by Nomura *et al.*,[25] all studies reported clearance of the bacterium in greater than 70% patients.

The effects of eradication therapy on platelet count were evaluable in 825 (82.7%) of 998 *H. pylori*-infected patients with primary ITP. As expected, a high degree of variance was observed (heterogeneity χ^2 = 115.600, p < 0.001; I^2 = 86.3%), with responses ranging from 7% in the US study by Ahn *et al.*[30] to 100% in the Japanese study Nomura *et al.* (Table 5-2).[25] Egger's test revealed no evidence of significant publication bias (p = 0.167), and although a plot of study precision versus response failed to form a funnel shape, a symmetric spread around the pooled response was nevertheless noted, with greater divergence evident among studies possessing greater standard error (Figure 5-3). A decision was therefore made to pool response data across studies.

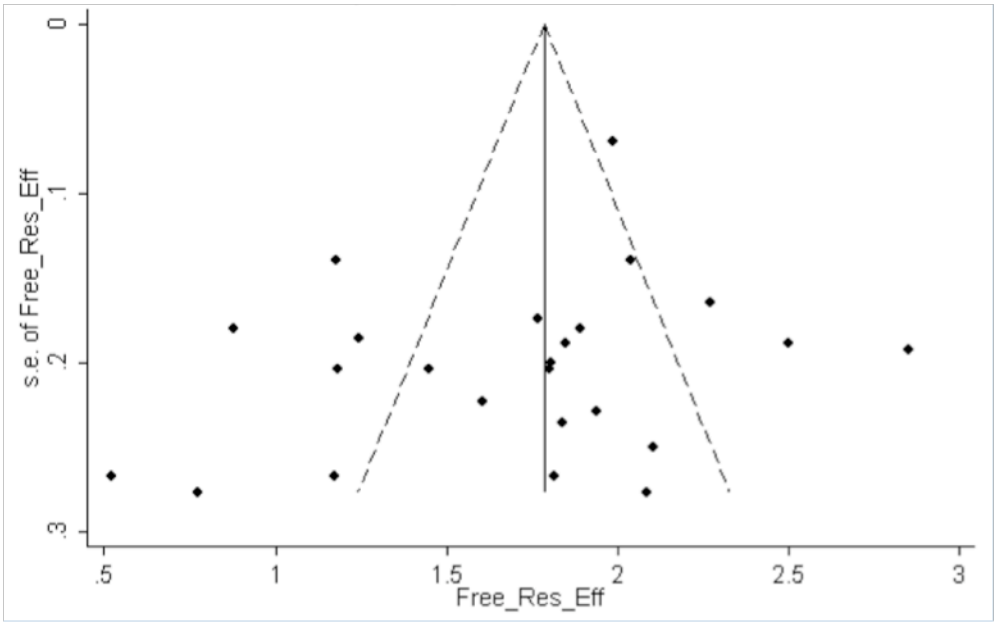

Figure 5-3: A funnel plot compares the effect size of each study (Freeman-Tukey arcsin square root transformed proportion of the response) against a measure of precision (its standard error). Studies with a smaller sample size (*i.e.*, precision) should have a larger random error and, thus, spread when graphed. In the absence of publication bias, then, effect estimates from smaller studies should have a larger, but symmetric, spread around the pooled effect.

Table 5-2: Results of *H. pylori* Eradication

Author (Year)	Successful Eradication N *(%)*	Count Before Eradication Mean ± SD[§] *x 10⁹/L*	Count After Eradication Mean ± SD *x 10⁹/L*	Response N *(%)*	Complete Response N *(%)*	Follow-up Time Median *Months*	Relapse N
Gasbarrini *et al.* (1998)	8 *(73)*	95 ± 39	140 ± 34	NA	NA	4	NA
Jarque *et al.* (2001)	23 *(72)*	58 ± 24	65 ± 32	3 *(13)*	NA	21	2
Kohda *et al.* (2002)	19 *(100)*	67 ± 54	120 ± 50	12 *(63)*	12 *(63)*	15	0
Hino *et al.* (2003)	18 *(86)*	37 ± 21	67 ± 54	8 *(44)*	6 *(33)*	38	NA
Hashino *et al.* (2003)	13 *(93)*	58 ± 30	99 ± 56	9 *(69)*	8 *(62)*	15	1
Ando *et al.* (2003)	27 *(93)*	56 ± 24	93 ± 49	16 *(59)*	13 *(48)*	11	1
Michel *et al.* (2004)	14 *(93)*	32 ± 15	66 ± 98	4 *(29)*	2 *(14)*	12	1
Takahashi *et al.* (2004)	13 *(87)*	40 ± 27	101 ± 86	7 *(54)*	6 *(46)*	4	NA
Sato *et al.* (2004)	27 *(84)*	54 ± 17	110 ± 21	15 *(56)*	NA	12	0
Ando *et al.* (2004)	15 *(88)*	49 ± 26	168 ± 43	10 *(67)*	10 *(67)*	24	0
Nomura *et al.* (2004)	12 *(43)*	29 ± 6	78 ± 11	12 *(100)*	NA	NA	NA
Veneri *et al.* (2005)	32 *(94)*	57 ± 23	122 ± 33	18 *(56)*	NA	24	1
Inaba *et al.* (2005)	25 *(100)*	52 ± 26	NA	11 *(44)*	NA	NA	0
Stasi *et al.* (2005)	52 *(100)*	42 ± 25	129 ± 61	16 *(31)*	13 *(25)*	25	6
Fujimura *et al.* (2005)	161 *(78)*	NA[**]	NA	88 *(55)*	34 *(21)*	12	NA
Suzuki *et al.* (2005)	22 *(88)*	55 ± 27	114 ± 90	6 *(27)*	6 *(27)*	6	NA
Suvajdzic *et al.* (2006)	23 *(77)*	68 ± 33	84 ± 45	6 *(26)*	NA	18	0
Ahn *et al.* (2006)	15 *(100)*	72 ± 45	69 ± 65	1 *(7)*	NA	NA	1
Sayan *et al.* (2006)	18 *(90)*	39 ± 16	100 ± 63	11 *(61)*	8 *(44)*	11	0
Asahi *et al.* (2006)	26 *(100)*	35 ± 13	114 ± 61	16 *(62)*	16 *(62)*	>12	0
Kodama *et al.* (2007)	44 *(85)*	40 ± 29	NR	27 *(61)*	8 *(18)*	NA	NA
Campuzano-Maya *et al.* (2007)	26 *(90)*	57 ± 38	198 ± 98	21 *(81)*	21 *(81)*	12	NA
Estrada-Gomez *et al.* (2007)	14 *(100)*	NA	NA	2 *(14)*	2 *(14)*	5	1
Satake *et al.* (2007)	23 *(92)*	NA	NA	13 *(57)*	12 *(52)*	25	0
Emilia *et al.* (2007)	34 *(89)*	41 ± 24	134 ± 96	25 *(74)*	22 *(65)*	43	1

[§] Standard deviation
[**] Not available

Response to Eradication Therapy

Although platelet counts were most commonly assessed one month following the completion of eradication therapy, data suggested that responses might have transpired earlier. Hino *et al.*,[18] for example, observed recovery as early as 3 days following eradication therapy while Asahi *et al.*[32] noted increased platelet counts in roughly half of responders after one week. In the study by Emilia *et al.*[36] initial platelet count improvement most commonly occurred 2 weeks after therapy.

Using the criteria adopted for this review, CR and response were demonstrated in 42.7% (95% CI, 31.8%-53.9%) and 50.3% (95% CI, 41.6%-59.0%) of 696 patients, respectively. Restriction of these analyses to patients with a baseline platelet count less than 30 x 10^9/L (15 studies, 222 eradicated patients) yielded a CR of 20.1% (95% CI, 13.5%-26.7%) and a response of 35.2% (95% CI, 28.0%-42.4%). Notably, regression analysis indicated a significant positive correlation (r = 0.351, p = 0.018) between the prevalence of *H. pylori* infection and platelet response to eradication therapy (Figure 5-4).

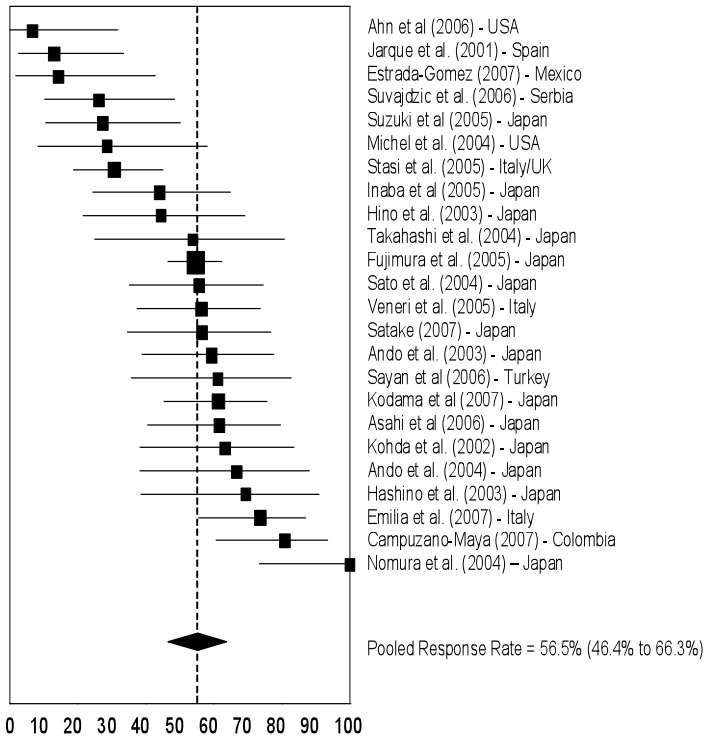

Figure 5-4: Response to *H. pylori* eradication. The solid boxes and horizontal lines are representative of the observed response and 95% confidence intervals for each study, respectively; the dimensions of the boxes are proportional to the relative weight given to each study. The dashed vertical line reveals the weighted mean response for the studies (56.5%).

A significant response among 15 (32.6%) patients in whom the eradication of infection was not successful was reported in the retrospective study by Fujimora *et al.*[35] Such a response was, however, not observed in 41 uninfected patients from 6 studies[18,21,22,24,27,32] who had also received eradication therapy.

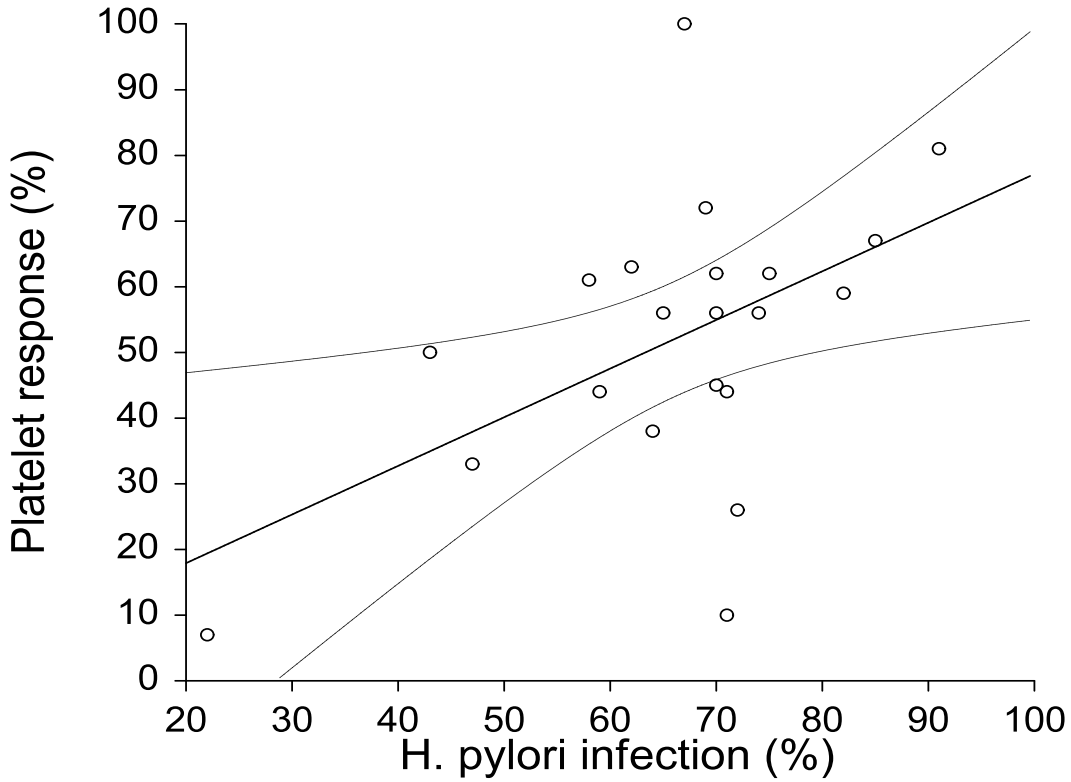

Figure 5-5: Correlation between prevalence of infection and response. A significant positive correlation (simple Pearson coefficient r = 0.3510, p = .0182) was observed.

Response Duration

The duration of response was not reported in 4 studies.[25,27,33,38] In the remaining 20 studies, the median duration of follow-up for responders ranged from 4 to 43.5 months. Responses were ongoing at the time of the report for greater than 90% of responders. In the study with the longest follow-up, that by Emilia *et al.* [36] (median: 43.5 months [range 18-90 months]), only one (4.3%) responder relapsed 7 or more months after eradication.

Predictors of a response to H. pylori eradication therapy

Clinical variables were evaluated for possible associations with response in 13 studies, encompassing 511 patients with eradicated *H. pylori* infections. As with response itself, postulated predictors of response proved heterogeneous. The most commonly reported predictor was duration of primary ITP, with studies by Kodama *et al.*,[33] Stasi *et al.*,[28] and Fujmura *et al.*[37] all documenting significantly shorter disease duration among responders. A genetic predisposition to response among carriers of the HLA–DQB1*03 allele was reported by Veneri *et al.*[26] while studies by Ando *et al.*[20] and Sato *et al.*[23] uncovered response associations with prior and concomitant corticosteroid therapy, respectively. These findings, however, have yet to be reproduced.

Discussion

Results from the meta-analysis revealed a CR and response in 42.7% (95% CI, 31.8%-53.9%) and 50.3% (95% CI, 41.6%-59.0%), respectively, in patients in whom *H. pylori* had been successfully eradicated. Responses were noted to persist in greater than 90% of initial responders over median follow-up times, which ranged from 4 to 43.5 months. While responses appeared lower in patients with more severe thrombocytopenia, participant-level data for studies by Nomura *et al.*[25] and Satake *et al.*[35] were not available. As these studies documented high responses among cohorts with low mean baseline platelet counts, the theory that eradication therapy is relatively ineffective in elevating platelet counts among markedly thrombocytopenic patients could not be reliably assessed.

As Crowther *et al.*[39] note, the heterogeneity of observed responses would typically preclude quantitative pooling of results across studies. However, the similar methodologies employed by the studies, the lack of significant publication bias, and the comparable number of studies with similar weight at both extremes of the response spectrum suggested that pooling was justified, particularly if coupled with subgroup analyses to explore possible sources of heterogeneity.

As shown in Figure 5-5, such analyses demonstrated a direct correlation between response and the prevalence of *H. pylori* infection. One plausible explanation for this finding may relate to the cytotoxin-associated gene A (CagA)-positive strain of *H. pylori*. CagA, a virulence factor protein, has been identified as a possible pathogenic candidate for primary ITP by Franceschi *et al.*[40] and Takahasi *et*

al.,[22] who demonstrated its capacity to molecularly mimic platelet antigens; their investigations further documented associations between *H. pylori* eradication and both the disappearance of anti-CagA antibodies and an increase in platelet counts.[22,40] CagA-positive strains of *H. pylori* vary depending upon geographic location. For example, in Japan, where prevalence and response to eradication therapy are generally high, most *H. pylori* strains express CagA, whereas the proportion of CagA-positive strains in Western countries is h lower.[41,42] While this theory appears promising, considerable work is still needed to evaluate it formally.

The failure to include non-English language articles and conference abstracts served as source of potential bias in this study. Both types of publications are more likely than prominent journals to include investigations yielding null findings.[43] As many of these studies are methodologically rigorous,[44] their exclusion may have resulted in skewed findings. Only one PubMed-indexed, non-English language cohort study of 15 of more patients with primary ITP was excluded from this investigation. It is, however, unknown how many conference abstracts may have met the remaining stringent inclusion criteria. The potential for publication bias in this regard must be considered.

Similarly, confounding may have also been present, as a substantial proportion of patients were previously or concurrently treated with immunosuppressive agents at the time of eradication therapy. These treatments may have contributed in part to observed increases in platelet counts. However, in a recent follow-up meta-analysis, Arnold *et al.*[45] reported 14.5-fold higher odds (95% CI, 4.2-83.0) of *H. pylori*-positive patients achieving a response from eradication therapy than *H. pylori* negative patients, suggesting that the high response observed in this study was tied to removal of the *H. pylori* bacterium from the body.

In conclusion, given the low costs, the non-invasiveness of diagnostic methods, and favourable toxicity profile of eradication therapy compared to conventional ITP treatments, the results of this meta-analysis support routine detection and eradication of *H. pylori* in adults with primary ITP in populations with a high prevalence of infection. A large international RCT will be required to determine whether the results of this meta-analysis can be replicated in an environment free from substantial bias and confounding and to assess the results of eradication therapy relative to an existing standard of care (*e.g.*, corticosteroids). Such an RCT

would ideally be coupled with molecular investigations, which would further explore possible pathogenic mechanisms by which *H. pylori infection* may trigger primary ITP.

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Chapter 6: Health-related lifestyle among patients with primary ITP

Summary

In an effort to complement ongoing health-related quality of life (HRQoL) research, a 43-question, closed-field questionnaire was used to identify health-related lifestyle concerns among patient members of the UK Adult ITP Support Association. In total, 790 patients, 696 (88.1%) adults and 94 (11.9%) children, returned completed questionnaires. Elective surgery had been delayed due to a low platelet count in 31.3% of adult patients, and a further 30.2% experienced difficulty obtaining travel insurance. Notably, 12.5% of all patients reported "always" or "often" missing work or school due to fatigue. These results highlight several promising and necessary avenues for patient advocacy and future HRQoL research.

Introduction

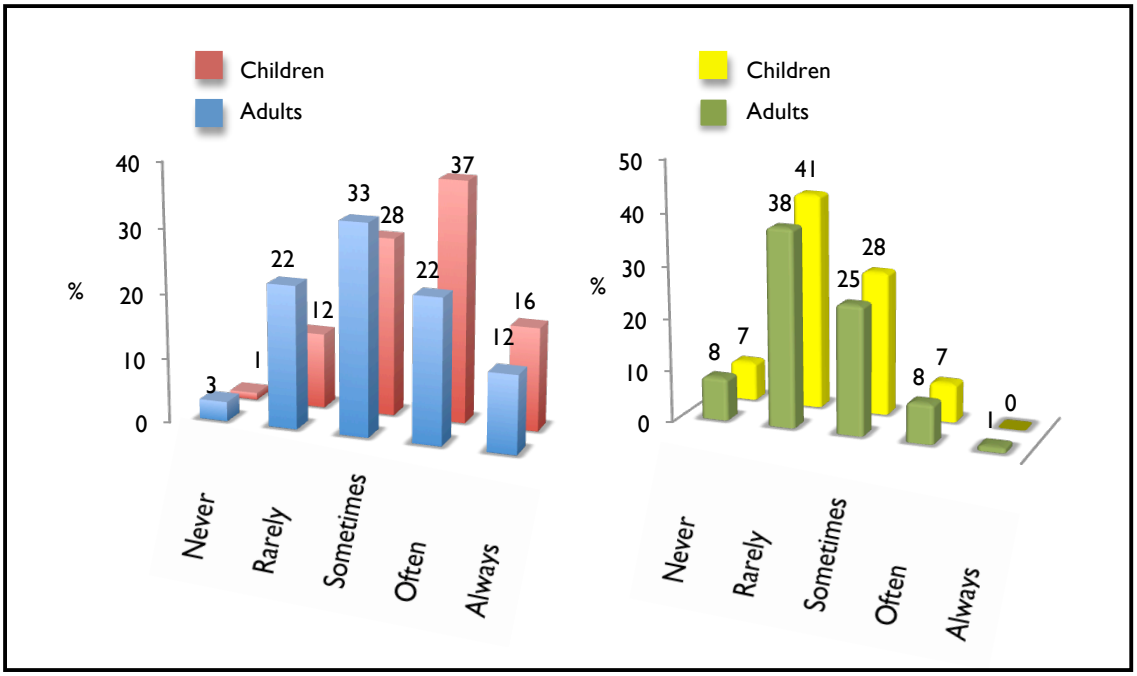
As discussed in Chapter 1, patients with primary ITP have historically been subject to considerable lifestyle restrictions. Investigation into the impact of these restrictions, of highly visible bleeding manifestations, and of patient concerns over the side effects of treatment, though only recently begun,[1,2] has helped highlight the considerable burden posed by the disease.[3,4] To complement ongoing HRQoL research, the UK ITP Support Association conducted a questionnaire-based survey with the aim of identifying health-related lifestyle concerns among its membership.

Methods

In collaboration with ITP specialists, a 43-question, closed-field questionnaire was developed, addressing bruising and bleeding frequency; disease management; social engagement; work and school performance; and recreational activities. Questionnaires were mailed to members of the ITP Support Association (N = 1,767). Results from returned questionnaires were stratified by age (adults [> 16 years] and children) and platelet count at last follow-up, which served as a surrogate marker for disease severity (mild: > 50 × 10^9/L, moderate: 20-49 × 10^9/L, and severe: < 20 × 10^9/L). Differences between groups were evaluated using generalised Cochrane-Mantel-Haenszel tests and were restricted to fields completed by at least three-fourths of responders.

Results

In total, 790 (44.7%) completed surveys were received from 696 (88.1%) adults and 94 (11.9%) children with primary ITP. The female-to-male ratio was 2.2:1, with respondents reporting a 5.0-year median (range: 0.1-54.0 years) duration of disease. Mild, moderate, and severe disease was noted in 57.2%, 17.5%, and 15.4% of patients. Bruising occurred "always" or "often" in 37.4% of adults and 56.8% of children, while bleeding of similar frequency was noted in 10.6% of all patients (Figure 6-1). Adults were twice as likely as children (89.4% vs. 45.3%; $p < 0.001$) to have been prescribed an ITP-specific treatment.



(a) **(b)**

Figure 6-1: Frequency of bruising (a) and wet bleeding (b) in adults and children with primary ITP. A low frequency of wet bleeding was reported by respondents, supporting data from population-based studies.

Children were more likely than adults to experience frustration over activity restrictions (23.3% vs. 9.5% respectively; $p < 0.001$). However, the impact of primary ITP on healthcare, insurance coverage, and social engagement was more pronounced among adults; 31.3% of adults reported having surgery delayed due to a low platelet count, and 30.2% experienced difficulty obtaining travel insurance. Although the incidence of bleeding manifestations were comparable between men and women, significant gender disparities were noted with regard to suspicion of physical violence (women: 7.1% vs. men: 1.6%; $p = 0.001$) and efforts at bruise concealment (women: 13.5% vs. men: 3.2%; $p < 0.001$) (Table 6-1). Notably, 12.5% of all patients with primary ITP reported "always" or "often" missing work or school due to fatigue. These absences were not significantly associated with disease severity ($p = 0.301$).

Table 6-1: Health-Related Lifestyle Survey Summary Results

QUESTION	POSITIVE RESPONSE %				
	TOTAL	Adults N = 696		Children N = 94	
	N = 790	Male N = 199	Female N = 497	Male N = 51	Female N = 43
Have you ever taken prescribed drugs for your primary ITP?	**84.9** *741*[*]	**89.3** *187*	**89.1** *479*	**45.0** *40*	**45.7** *35*
Have you ever tried alternative therapies or vitamins and minerals supplements for your primary ITP?	**29.4** *625*	**24.2** *157*	**31.4** *392*	**24.4** *41*	**37.1** *35*
Have you had difficulty obtaining or been refused travel insurance?	**28.8** *608*	**29.2** *154*	**30.5** *383*	**10.0** *40*	**9.7** *31*
Have you ever had surgery postponed or delayed because of a low platelet count?	**29.5** *620*	**28.4** *158*	**30.4** *391*	**18.8** *39*	**12.8** *32*
Do you try to hide your bruises?[†]	**10.7** *756*	**3.2** *190*	**13.5** *475*	**10.0** *50*	**14.6** *41*
Are people ever suspicious that the bruises are a result of physical violence?[†]	**5.7** *750*	**1.6** *193*	**7.1** *468*	**5.9** *49*	**10.0** *40*
I get bothered because I cannot do the activities I like.[†]	**11.8** *726*	**10.1** *184*	**10.9** *456*	**23.9** *46*	**22.5** *40*
Have you ever been unable to go to work or school because of tiredness and fatigue?[†]	**12.5** *710*	**10.8** *186*	**14.3** *435*	**6.1** *49*	**10.0** *40*

[*] Total number of responses recorded for the given question.
[*] Questions with ordinal responses (*i.e.*, always, often, sometimes, never, and rarely) collapsed into dichotomous results (*i.e.*, yes and no) for the purposes of this table.

Discussion

This study represents one of the largest HRQoL investigations in primary ITP to date and strengthens recurrent reports of fatigue in patients. A pathological basis for its onset has not been determined and warrants further investigation.[2] The results of our study further demonstrate the need for HRQoL metrics to incorporate the effects of treatments on fatigue, bruising, and activity restriction. The ITP-PAQ and KIT do so, but our findings suggest that increased weighting of bruising and activity restriction may be merited in women and children, respectively.

The reported difficulty of adults in securing travel insurance prompts concern. Population-based studies have revealed a low incidence of major bleeding events in adults with primary ITP.[5] Dissemination of this information to insurers may be facilitated by patient support groups, which may help to make access to coverage more straightforward and affordable.

Finally, reports of delayed surgery in one-third of adults with primary ITP highlights a need for improved coordination of care between surgeons and haematologists. Surgery may often be safely performed in thrombocytopenic patients;[6] in this respect, an international consensus group of ITP specialists has recently published a table of recommended platelet counts for a variety of procedures.[‡]

While this large-scale survey provides useful insight into health-related lifestyle concerns among patients with primary ITP, its limitations warrant consideration. First, the questionnaire utilised was designed as a descriptive tool and is not a validated instrument. Second, the proportion of respondents (44.7%) was low, raising questions about the representativeness of the sample. It should be noted, however, that membership to the ITP Support Association was not restricted to patients with primary ITP; spouses and parents of patients comprised a minority population within the group. Although the exact number of non-patient members at the time of the survey was not known, it is reasonable to assume that the proportion of patient responders was significantly higher than 45%. Third, our use of last platelet count as a proxy for disease severity did not account for disease progression or treatment effects over time and may have therefore biased our observation of a non-significant association between disease severity and fatigue.

[‡] http://bloodjournal.hematologylibrary.org/cgi/data/blood-2009-06-225565/DC1/8

Despite these limitations, the results of our health-related lifestyle survey of patients with primary ITP in the UK successfully highlight several avenues for research, including demographically-dependent variable weighting in HRQoL metrics and the pathogenesis of primary ITP associated fatigue, while uncovering the need for greater dialogue between both patient support groups and insurers and haematologists and surgeons.

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Chapter 7: Thromboembolic events among adults with primary ITP

A study using the General Practice Research Database

Summary

The risk of thromboembolic events (TEs) in adults with primary ITP has been minimally investigated despite findings of increased susceptibility in other thrombocytopenic autoimmune conditions. The risk of TEs was therefore evaluated in adult patients with and without primary ITP in the United Kingdom General Practice Research Database (GPRD). In total, 1,070 adults (≥ 18 years) with coded records for primary ITP first referenced between January 1st 1992 and November 30th 2007 and having at least one year pre-diagnosis and 3 months post-diagnosis medical history were matched (1:4) with 4,280 ITP-free patients by age, sex, primary care practice, and pre-diagnosis observation time. The baseline prevalence and incidence rate of TEs were quantified, with comparative risk evaluated with multivariable (ITP treatment and co-morbid conditions) Cox proportional hazards models. Over a median time of 47.6 months (range: 3.0-192.5 months), adjusted hazard ratios of 1.58 (95% CI, 1.01-2.48), 1.37 (95% CI, 0.94-2.00), and 1.41 (95% CI, 1.04-1.91) were found for venous, arterial, and combined (arterial and venous) TEs in the primary ITP cohort relative to the ITP-free cohort, respectively. Further event categorisation revealed elevated incidence rates of each occurring venous TE subgroup among adults with primary ITP. These results illustrated an increased, consistent risk of venous TEs in adults with primary ITP.

Introduction

While efforts to understand disease progression in adults with primary ITP have understandably centred on site-specific haemorrhagic risks, further investigation into thromboembolic susceptibility may be warranted. Elevated rates of arterial and venous thrombembolic events (TEs) have been well documented in a series of autoimmune diseases, including idiopathic thrombotic thrombocytopenic purpura (TTP)[1] and systemic lupus erythematosus (SLE).[2] A retrospective study conducted by Aledort *et al.*[3] suggests that a heightened risk may be present in primary ITP. In their multi-centre cohort of 186 adults, a total of 18 TEs were recorded in 10 patients, of which 11 TEs (61.1%) occurred following the diagnosis of primary ITP. More recently, using administrative claims from a large health plan affiliated with the company i3 Drug Safety in the United States, Bennett *et al.*[4] reported a 6.9% cumulative incidence of TEs among adult patients with chronic, primary ITP over a median follow-up time of 15 months.[4] Knowledge of an association between primary ITP and TEs among adults, were one to exist, may influence the long-term management strategies. The objective of this study was to evaluate the risk of TEs among adult patients with and without primary ITP in the General Practice Research Database (GPRD).

Methods

Data Source

As discussed in Chapter 1, data from the GPRD are drawn from the computer systems of general practices throughout the UK and currently include information regarding diagnoses, prescriptions, referrals, outcomes, and laboratory results, together with basic demographic information, for approximately 6.4 million patients from over 480 centres. The database is population-based and representative of the age, sex, and geographic regions of the UK.[5] Inclusion is based on registration with a contributing general practice, rather than consultations, and there is no requirement for patients to be actively receiving treatment. Disease-associated data are stored in the GPRD using Oxford Medical Information System (OXMIS) or Read codes, which are cross-referenced to International Classification of Diseases (ICD-9) codes[6] (OXMIS code usage was primarily restricted to the period prior to the introduction of Read codes in 1997). The quality of entered data is continuously monitored by the MHRA, with practices failing to adhere to established standards excluded from

participation. GPRD coding has been subject to a number of validation studies, which have found it an accurate identification tool for a wide spectrum of conditions and diseases.[7-9]

Study Design and Population

A retrospective cohort design was adopted for the study. Data were collected for adult patients (age ≥ 18 years) with OXMIS or Read codes for primary ITP first referenced between January 1st, 1992 and November 30th, 2007 in the GPRD. Utilised codes (Read: 288599, D313000, D313012, 42P2.11 and OXMIS: 2871C) were determined by the study team, which included a physician with extensive experience managing adult patients with primary ITP in the NHS. Inclusion within the primary ITP cohort was restricted to code-identified patients with at least one year pre-diagnosis and three months post-diagnosis medical history. These criteria were applied to ensure sufficient information was available to determine a patient's baseline medical status and provide a minimum period of post-ITP follow-up. To define thrombocytopenia, a commonly utilised platelet count threshold of less than 150×10^9/L was selected a priori during protocol development in 2007, prior to publication of new consensus terminology recommendations published by the International Working Group on ITP[10] in 2009.

For comparative purposes, a non-case cohort was assembled and consisted of primary ITP-disease free adult patients from the GPRD matched at a ratio of 1:4 by age (5-year bands), gender, primary care practice, and pre-index observation time (1, 2, 3 & 4 years). Date of entry into the primary ITP cohort (index date) was defined as the date of diagnosis, and index dates for the primary ITP-disease free cohort were taken from their matched counterparts.

Outcomes

The outcomes of interest were TEs, grouped as venous, arterial, and combined (venous or arterial) TEs. Events were identified using OXMIS/Read codes and sub-grouped by deep vein thrombosis (DVT), pulmonary embolism (PE), portal vein thrombosis (PVT), other venous TEs, myocardial infarction (MI), unstable angina (UA), ischaemic stroke (IS), transient ischaemic attack (TIA), other arterial TEs, and unclassifiable TEs.

Exposures & Covariates

Primary ITP status comprised the principal exposure in the study. Additional covariates included ITP-treatment (oral corticosteroid usage, IVIg treatment, and splenectomy status), age (grouped as 18-39, 40-49, 50-59, 60-69, 70-79, 80-89 & ≥ 90 years), gender, and baseline co-morbid status (hypertension, diabetes, chronic renal failure, and prior TEs).

Follow-Up Time

Follow-up time for each patient extended from the date of cohort entry until censoring, disenrollment from the database (by the patient or contributing primary care practice), death, or end of the study period (31 December 2007). Censoring took place at the time of first event occurrence (*e.g.*, patients with a prior history of arterial but not venous TEs were excluded from arterial and combined TE rate analyses). Thus, only truly incident TEs were assessed.

Statistical Analyses

Incidence and Cox survival analyses were based on the follow-up time detailed above. For analyses of prevalence and cumulative incidence, TEs were counted over discrete, post-index intervals of 1-90 days, 91-180 days, 181-360 days, 361 days to 2.5 years, and greater than 2.5 years. Incidence rates (IRs) for venous, arterial, and combined TEs were reported within these intervals, with cumulative incidence estimates compiled over post-index periods of 180 days, 360 days, and 2.5 years.

Following inspection of logarithmic graphs of cumulative survival to verify assumptions of proportional hazards between the cohorts, unadjusted and adjusted (covariates: ITP-treatment and co-morbid conditions) hazard ratios (HRs) of venous, arterial, and combined TEs were calculated using Cox regression models on SAS 9.1.3 (Cary, North Carolina).

To explore the relationship between thrombocytopenic severity with incident TEs, post-hoc subgroup analyses were performed to calculate TE incidence rate ratios (IRRs) of primary ITP patients with baseline platelet counts (1) < 50 × 10^9/L, (2) 50-75 × 10^9/L, and (3) 75-150 × 10^9/L relative to the primary ITP disease-free cohort.

Ethics

The study protocol was approved by the Independent Scientific Advisory Committee of the GPRD for the UK Medicines and Healthcare Products Regulatory Agency.

Results

Study Population

Baseline characteristics of the primary ITP and primary ITP-disease free cohorts are illustrated in Table 7-1. Briefly, 1,070 and 4,280 adult patients with and without primary ITP were identified, respectively, and followed for a median time of 47.6 months (range: 3.0-192.5 months). The female-to-male ratio within the primary ITP cohort was 1.4:1, with platelet count data available for 694 (64.9%) patients.

Differences in the prevalence of several co-morbidities at baseline were noted between adult patients with and without primary ITP, including diabetes (100 [9.3%] v. 231 [5.4%], $p < 0.001$), chronic renal failure (30 [2.8%] v. 56 [1.3%], $p < 0.001$), previous venous TEs (64 [6.0%] v. 198 [4.6%], $p = 0.066$), and previous arterial TEs (105 [9.8%] v. 280 [6.5%], $p < 0.001$).

Oral corticosteroids had been used by 200 (18.7%) patients with primary ITP within a one-year period prior to study entry; a further 25 (2.3%) patients had already undergone splenectomy. By two years post-index, the proportion of oral corticosteroid-treated and splenectomised adult patients with primary ITP had climbed to 294 (37.7%) and 53 (6.8%) respectively. The GPRD did not, however, capture the administration of IVIg, an acute treatment administered in hospital care settings.

Table 7-1: Baseline Characteristics of the Study Population

Description	Primary ITP Cohort	Primary ITP-Disease Free Cohort	P Value Unmatched Variables
All patients	**1,070**	**4,280**	
Gender & Age-*Years* (%)			
Female	620 (57.9)	2,480 (57.9)	
18-39	179 (16.7)	716 (16.7)	
40-49	73 (6.8)	292 (6.8)	
50-59	97 (9.1)	388 (9.1)	
60-69	93 (8.7)	372 (8.7)	
70-79	101 (9.2)	404 (9.2)	
80-89	70 (6.5)	280 (6.5)	
≥ 90	7 (0.7)	28 (0.7)	
Male	450 (42.1)	1,800 (42.1)	
18-39	81 (7.6)	324 (7.6)	
40-49	58 (5.4)	232 (5.4)	
50-59	68 (6.4)	272 (6.4)	
60-69	72 (6.7)	288 (6.7)	
70-79	120 (11.2)	480 (11.2)	
80-89	45 (4.2)	180 (4.2)	
≥ 90	6 (0.6)	24 (0.6)	
Co-Morbid Conditions (%)			
Hypertension	297 (27.8)	1,110 (25.9)	0.226
Diabetes	100 (9.3)	231 (5.4)	< 0.001
Chronic Renal Failure	30 (2.8)	56 (1.3)	< 0.001
Platelet Count x 10^9/L (%)			
< 50	173 (16.2)	1 (0)	< 0.001
50-75	136 (12.7)	0 (0)	
75-150	246 (23.0)	34 (0.8)	
No Data Available	376 (35.1)	3,152 (73.6)	
ITP-Specific Treatment (%)			
Past Year OCS	200 (18.7)	180 (4.2)	< 0.001
Past Year IVIg Use	0 (0)	1 (~0.0)	0.617
Splenectomy	25 (2.3)	3 (0.1)	< 0.001
Thromboembolic Event History (%)			
Venous TEs	64 (6.0)	198 (4.6)	0.066
Arterial TEs	105 (9.8)	280 (6.5)	< 0.001
Combined TEs	165 (15.4)	453 (10.6)	< 0.001

Table 7-2: Incidence Rates of Venous & Arterial TEs by Code

Codes	Primary ITP Cohort			Primary ITP-Disease Free Cohort		
	Patients At Risk	Events	Incidence Rate *(95% CI)*	Patients At Risk	Events	Incidence Rate *(95% CI)*
Venous TEs						
Idiopathic Codes	932	29	**67.47 *(45.18, 96.90)***	4,082	82	**42.45 *(33.76,52.70)***
Read Idiopathic Thrombocytopenic Purpura D313000 & D313012 ITP-Idiopathic Thrombocytopenic Purpura 42P2.11 OXMIS Idiopathic Thrombocytopaenia 2871C						
Autoimmune Codes	74	2	**56.06 *(6.79, 202.50)***			
Read Autoimmune Thrombocytopenia D313.12						
Arterial TEs						
Idiopathic Codes	897	40	**94.28 *(67.36 128.93)***	4,000	128	**67.40 *(56.23,80.14)***
Read Idiopathic Thrombocytopenic Purpura D313000 & D313012 ITP-Idiopathic Thrombocytopenic Purpura 42P2.11 OXMIS Idiopathic Thrombocytopaenia 2871C						
Autoimmune Codes	68	4	**124.75 *(33.99, 319.40)***			
Autoimmune Thrombocytopenia 288599						

Cumulative Incidence & Incidence Rates

The cumulative incidence of first venous, arterial and combined TEs during the study was 2.9%, 4.1%, and 6.1% in the primary ITP cohort and 1.9%, 3.0%, and 4.6% in the primary ITP-disease free cohort, respectively.

IRs* of venous (IR: 66.59 [95% CI, 45.25-94.52]) vs. 42.45 [95% CI, 33.76-52.70]) and arterial TEs (IR: 96.42 [95% CI, 70.06-129.45]) vs. 67.40 [95% CI, 56.23-80.14]) were elevated among patients with primary ITP, an increased risk seen across the autoimmune (Read: 42P2.11) and idiopathic ([Read: D313.12, D313000 & D313012] & OXMIS [2871C]) coding strata (Table 7-2). Sub-grouping shown in Figure 7-1 depicts increased rates of MI (IR: 52.21 vs. 22.73), UA (IR: 24.32 vs. 13.36), other arterial TEs (IR: 7.98 vs. 1.47), DVT (IR: 22.42 vs. 11.43), PE (IR: 16.11 vs. 4.93), and other venous TEs (IR: 37.44 vs. 30.93). Overlap was noted between the 95% CIs of the latter five sub-group estimates for the two cohorts. No cases of PVT were identified within the study population during the defined follow-up period.

Results from the stratification of venous and arterial TEs by baseline characteristics, highlighted in Tables 7-3 and 7-4, respectively, demonstrated noticeable disparities in the IRRs of both types of events in women and men, in patients with and without a past history TEs, and in patients taking oral corticosteroids.

Further subgroup analyses of combined TEs by baseline platelet count suggest the possibility of a direct relationship between disease severity and thrombosis as seen in Figure 7-2. Restriction of the primary ITP cohort to adult patients with presenting counts less than 100 × 10^9/L, a threshold recently advocated by the International Working Group on ITP[10] to exclude asymptomatic, mildly thrombocytopenic patients from disease categorisation, resulted in an elevated IRR of 1.55 (95% CI, 0.97-2.43) for combined TE. Moreover, IRR point estimates for combined TEs were increasingly elevated for moderately (50-75 × 10^9/L: 1.50 [95% CI, 0.67-3.39]) and severely (< 50 × 10^9/L: 1.74 [95% CI, 0.95-3.19]) thrombocytopenic adult patients.

* Expressed per 10,000 person-years

Table 7-3: Stratified incidence rates of venous TEs

Description	Primary ITP Cohort			Primary ITP-Disease Free Cohort			Incidence Rate Ratio (95% CI)
	Patients	Events	Incidence Rate (95% CI)	Patients	Events	Incidence Rate (95% CI)	
All patients	**1,006**	**31**	**66.59 (45.25,94.52)**	**4,082**	**82**	**42.45 (33.76,52.70)**	**1.57 (1.04,2.37)**
Gender & Age *(Years)*							
Female	579	20	71.40 *(43.61,110.27)*	2,362	53	45.67 *(34.21,59.73)*	1.56 *(0.93,2.62)*
Female; 40-49	69	2	47.01 *(5.69-169.80)*	285	3	18.97 *(3.91,55.44)*	2.48 *(0.41-14.83)*
Female; 50-59	83	3	74.92 *(15.45,218.96)*	375	5	25.58 *(8.31,59.69)*	2.93 *(0.70,12.26)*
Female; 60-69	84	3	71.14 *(14.67,207.91)*	359	11	58.08 *(29.00,103.93)*	1.22 *(0.34,4.39)*
Female; 70-79	92	5	126.93 *(41.21,296.22)*	369	15	87.53 *(48.99,144.36)*	1.45 *(0.53,3.99)*
Female; 80-89	68	2	92.54 (11.21,334.30)	242	11	134.67 (67.22,240.95)	0.69 *(0.15,3.10)*
Male	427	11	59.33 *(29.62,106.16)*	1,720	29	37.62 *(25.19,54.02)*	1.58 *(0.79,3.16)*
Male; 40-49	56	1	35.76 *(0.91,199.24)*	227	2	19.22 *(2.33,69.42)*	1.86 *(0.17,20.52)*
Male; 50-59	63	3	92.53 *(19.08,270.42)*	266	6	43.61 *(16.01,94.93)*	2.12 *(0.53,8.48)*
Male; 60-69	66	0		276	8	59.66 (25.76,117.55)	
Male; 70-79	113	3	70.07 *(14.45,204.77)*	437	11	57.21 *(28.56,102.37)*	1.22 *(0.34,4.39)*
Male; 80-89	43	3	274.45 *(56.60,802.07)*	170	1	20.91 (0.53,116.53)	13.12 *(1.36,126.16)*
Baseline Platelet Count *(x10⁹/L)*							
< 50	164	1	15.08 *(0.38,84.00)*	1	0		
50-75	128	2	48.35 *(5.86,174.67)*	0	0		
75-150	233	5	61.80 *(20.07,144.22)*	29	1	95.92 *(2.43,534.41)*	0.64 *(0.08,5.52)*
No Data Available	354	20	87.56 *(53.49,135.24)*	3,030	63	39.95 *(30.70,51.11)*	2.19 *(1.33,3.62)*
ITP-Specific Treatment							
Corticosteroid Yes	474	14	60.37 *(33.00,101.29)*	641	16	51.16 *(29.24,83.08)*	1.18 *(0.58,2.42)*
Corticosteroid No	532	17	72.77 *(42.39,116.52)*	3441	66	40.77 *(31.53,51.87)*	1.78 *(1.05,3.04)*
IVIg Yes	2	0		8	0		
IVIg No	1004	31	66.93 *(45.48,95.00)*	4074	82	42.56 *(33.85,52.83)*	1.57 *(1.04,2.38)*
Splenectomy Yes	79	3	67.96 *(14.01,198.60)*	3	0		
Splenectomy No	927	28	66.45 *(44.16,96.04)*	4079	82	42.48 *(33.79,52.73)*	1.56 *(1.02,2.40)*
Thromboembolic Event History							
Prior TEs Yes	101	4	117.03 *(31.89,299.65)*	255	5	49.38 *(16.03,115.23)*	2.37 *(0.64,8.83)*
Prior TEs No	905	27	62.60 (41.25,91.08)	3,827	77	42.07 *(33.20,52.58)*	1.49 *(0.96,2.31)*

Table 7-4: Stratified incidence rates of arterial TEs

Description	Primary ITP Cohort			Primary ITP-Disease Free Cohort			Incidence Rate Ratio (95% CI)
	Patients	Events	Incidence Rate (95% CI)	Patients	Events	Incidence Rate (95% CI)	
All patients	**965**	**44**	**96.42 *(70.06,129.45)***	**4,000**	**128**	**67.40 *(56.23,80.14)***	**1.43 *(1.02,2.02)***
Gender & Age *(Years)*							
Female	583	20	69.33 *(42.35,107.08)*	2375	71	60.34 *(45.52,74.05)*	1.15 *(0.70,1.89)*
Female; 40-49	73	0		291	4	24.74 *(6.74,63.35)*	
Female; 50-59	92	2	44.78 *(5.42,161.75)*	379	6	30.12 *(11.05,65.55)*	1.49 *(0.30,7.37)*
Female; 60-69	91	7	149.80 *(60.23,308.64)*	362	16	83.92 *(47.97,136.29)*	1.78 *(0.73,4.34)*
Female; 70-79	89	6	160.41 *(58.87,349.14)*	365	24	138.23 *(88.56,205.67)*	1.16 *(0.47,2.84)*
Female; 80-89	55	3	164.83 *(33.99,481.71)*	236	19	240.70 *(144.92,375.88)*	0.68 *(0.20,2.31)*
Male	382	24	142.98 *(91.61,212.74)*	1625	57	78.89 *(59.75,102.22)*	1.81 *(1.12,2.92)*
Male; 40-49	56	0		227	5	48.91 (15.88,114.14)	
Male; 50-59	62	3	97.56 *(20.12,285.12)*	262	7	51.49 *(20.70,106.09)*	1.89 *(0.49,7.33)*
Male; 60-69	61	9	353.18 *(161.49,670.44)*	251	6	50.89 *(18.67,110.76)*	6.94 *(2.47,19.50)*
Male; 70-79	92	9	260.52 *(119.13,494.55)*	400	27	158.28 *(104.31,230.29)*	1.65 *(0.77,3.50)*
Male; 80-89	29	2	247.53 *(29.98,2,894.15)*	144	11	268.17 *(133.87,479.83)*	0.92 *(0.20,4.16)*
Baseline Platelet Count *(x10⁹/L)*							
< 50	156	10	161.54 *(77.46,297.07)*	1	0		
50-75	119	6	156.60 *(57.47,340.85)*	0	0		
75-150	219	5	66.23 *(21.50,154.55)*	27	0		
No Data Available	348	19	81.32 *(48.96,126.99)*	2,995	89	57.15 *(45.90,70.33)*	1.42 *(0.87,2.33)*
ITP-Specific Treatment							
Corticosteroid Yes	448	22	96.51 *(60.48,146.11)*	626	23	74.28 *(47.09,111.46)*	1.30 *(0.72,2.33)*
Corticosteroid No	517	22	96.34 *(60.38,145.86)*	3,374	105	66.06 *(54.03,79.97)*	1.46 *(0.92,2.31)*
IVIg Yes	2	0		8	0		
IVIg No	963	44	96.72 *(70.28,129.84)*	3,992	128	67.57 *(56.37,80.34)*	1.43 *(1.02,2.02)*
Splenectomy Yes	81	5	113.41 (36.82,264.66)	3	0		
Splenectomy No	884	39	94.61 *(67.28,129.33)*	3,997	128	67.45 *(56.27,80.19)*	1.40 *(0.98,2.01)*
Thromboembolic Event History							
Prior TEs Yes	60	6	261.81 *(96.08,569.84)*	173	12	168.31 *(86.97,294.00)*	1.56 *(0.58,4.14)*
Prior TEs No	905	38	87.68 *(62.05,120.35)*	3,827	116	63.46 *(52.44,76.12)*	1.38 *(0.96,1.99)*

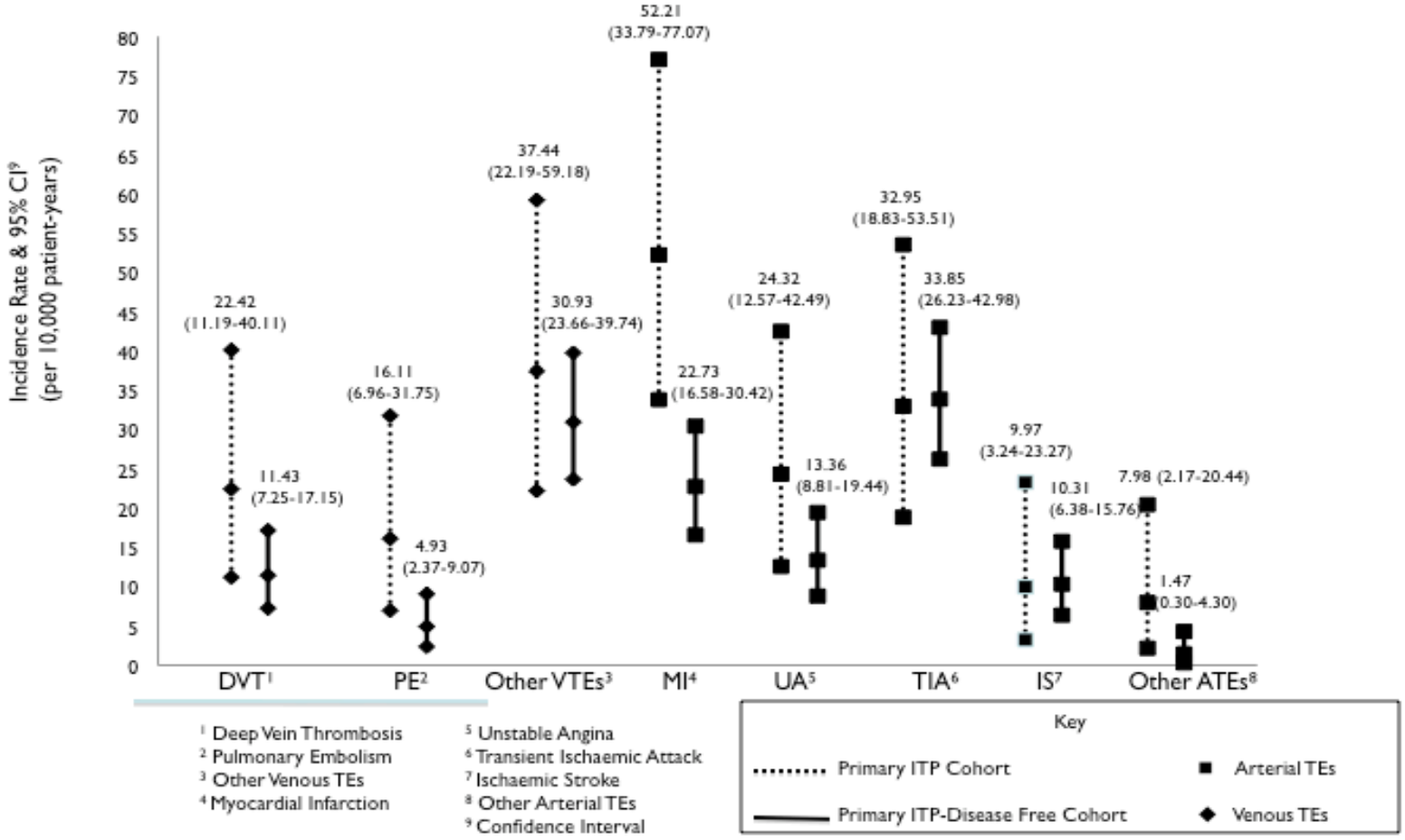

Figure 7-1: Incidence rates of venous & arterial TEs by subgroups. The incidence rates of deep vein thrombosis, pulmonary embolism, and other venous TEs were elevated in adults with primary ITP versus adults without primary ITP. No portal vein thromboses were observed over the course of follow-up.

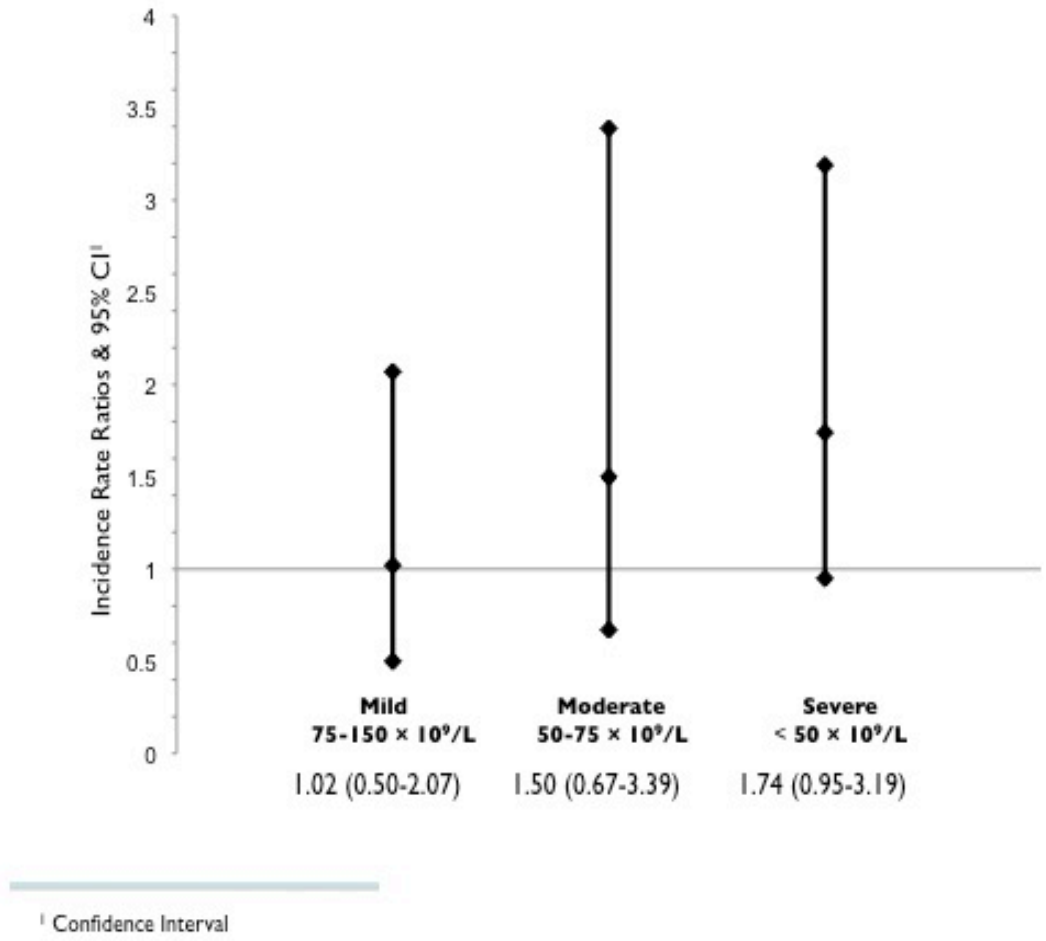

Figure 7-2: Incidence rate ratios of combined TEs among subgroups of the primary ITP cohort relative to the non-primary ITP cohort. A step-wise increase in incidence rate ratios was noted between patients with mild, moderate, and severe baseline thrombocytopenia, respectively.

Hazard Ratios

Unadjusted HRs of 1.58 (95% CI, 1.05-2.39), 1.42 (95% CI, 1.01-2.00), and 1.42 (95% CI, 1.07-1.88) were obtained for venous, arterial, and combined TEs, respectively. Adjustment for ITP-treatment (oral corticosteroid usage, IVIg treatment, and splenectomy) and co-morbid status (hypertension, diabetes, chronic renal failure, and history of prior TEs) altered these ratios only slightly, resulting in HRs of 1.58 (95% CI, 1.01-2.48), 1.37 (95% CI, 0.94-2.00), and 1.41 (95% CI, 1.04-1.91), respectively.

Discussion

Derived from population-based data,[5] the results of this study provide evidence of an increased risk of venous TEs among adults with primary ITP. An IRR of 1.57 (95% CI, 1.04-2.37) was observed between the primary ITP and non-primary ITP cohorts, with multivariable Cox regression modelling yielding an adjusted, significant HR of 1.58 (95% CI, 1.01-2.48). The IRs of each occurring venous TE subgroup, moreover, were higher in adults with primary ITP, demonstrating a consistency of effect (Figure 7-1). While evidence of an elevated risk of arterial TEs was similarly present, it was admittedly weaker, with modelling revealing an adjusted, non-significant HR of 1.37 (95% CI, 0.94-2.00). Owing to increasing challenges to the prevailing theory of distinct aetiologies for venous and arterial TEs,[12,13] further analyses were conducted on the risk of combined TEs, with data supporting a significantly increased hazard (adjusted HR = 1.41 [95% CI, 1.04-1.91]) in patients with primary ITP.

An initial concern of this investigation centred on the accuracy of the OXMIS and Read codes used to identify adults with primary ITP in the GPRD. In a recently published study, Schoonen *et al.*,[8] reported a very high positive predictive value (PPV, 91% [95% CI, 84-96%]) of a collection of 9 such codes. The following 4 (44.4%) codes from their investigation were, however, non-specific and, as a result, excluded from this study: 1) Evans syndrome (Read: D313.11), 2) platelet count (OXMIS: L 146N), 3) platelet count (Read: 42P..00), and 4) platelet count, nos (Read: 42PZ.00)). It is therefore likely the PPV of codes adopted for this investigation was commensurate, if not higher, than 91%.

The existence of two coding vocabularies (OXMIS and Read) and multiple codes for the same medical concept within these vocabularies raised an additional question as to whether patients labelled with one of the 5 codes selected to identify adult patients with primary ITP were systematically different from those classed under another. To investigate, IRs of venous and arterial TEs were calculated for the two primary classes of primary ITP codes, the autoimmune (Read: 42P2.11) and idiopathic ([Read: D313.12, D313000 & D313012] and OXMIS [2871C]) codes.* Similar estimates across both strata suggest that no appreciable differences were present (Table 7-2).

Two limitations of this study should be noted. First, the available number of adults with primary ITP in the GPRD may have hampered the power of the

investigation to detect a significant association between primary ITP and arterial TEs. Although 1,070 adult patients were included, making this study one of the largest investigations into primary ITP to date, pre-investigation power calculations revealed the need for 8,120 patients with primary ITP to detect a twofold increase in the estimated annual incidence of arterial TEs in the Western world.[21†]

Second, the paucity of available platelet counts and IVIg usage reflected a general limitation of the GPRD in capturing specific, hospital-based data. However, as numerous investigative teams have shown for a variety of conditions, including primary ITP,[8,9,11,12] this limitation nevertheless does not diminish the accuracy of the data that were captured.

Importantly, the uncovered associations do not in themselves implicate primary ITP as a causative agent for venous and combined TEs. Hospitalisation, for instance, may have been more common among the primary ITP cohort and is itself a widely recognised, independent risk factor for venous TEs.[13,14] Similarly, while significantly increased hazards of venous and combined TEs persisted following adjustment for co-morbid conditions and ITP-treatment, it is possible that treatment modalities other than those included (or adequately captured) may have played a role in the creation of a pro-thrombotic environment.

While knowledge of an elevated risk of venous TEs among adults with primary ITP may support increased use of thromboprophylactic treatment in patients at lower risk of haemorrhage, further work is first needed to confirm the uncovered association and to examine whether evidence exists implicating a causative role for ITP pathogenesis in thrombus formation, topics explored at greater length in Chapters 8 and 9, respectively. As with future investigations into SNP associations with primary ITP (Chapter 3), follow-up studies would benefit from the development of an international registry, which would enable suitably powered evaluation of a wider range of variables (*i.e.*, hospital-based data).

† At 80% 1-β and 5% α.

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Chapter 8: Thromboembolic events among adults with primary ITP

A study using the United Kingdom Adult ITP Registry

Summary

While an increased risk of venous thromboembolic events (TEs) was found among adults with primary ITP in the GPRD, the external validity of this finding was unclear. In particular, it was uncertain whether code-identified patients were representative of adults under active management for primary ITP. Data from the United Kingdom Adult ITP Registry and population-based studies in the medical literature were therefore used to conduct a retrospective cohort study on the age- and sex-adjusted risk of TEs in Registry participants relative to the general adult population. Increased, though non-significant, risks were observed in the primary ITP cohort for each arterial and venous TE subgroup evaluated. The pooling of these estimates via random-effects meta-analyses yielded significant standardised rate ratios (SSRs) for venous (2.43 [95% CI, 1.01-5.83]), arterial (2.45 [95% CI, 1.48-4.06]), and combined TEs (2.44 [95% CI, 1.58-3.79]), respectively, illustrating an over two-fold, consistent risk of TEs in actively managed adults with primary ITP. Considerable work is required to ascertain whether these associations may reflect a causal relationship between primary ITP pathogenesis and TE onset.

Introduction

As discussed at the conclusion of Chapter 7, the finding of significantly increased risks of combined and venous TEs among adults with primary ITP in the GPRD warrants further investigation. Although results from the study by Schoonen *et al.*[1] support the accurate classification of patients in the primary ITP cohort, it remains unclear whether they were representative of patients under active management. Validation of GPRD findings in such a representative population is required prior to discussion of the potential impact these associations may have on disease management. The objective of this study was to evaluate the risk of TEs among patients in the UK Adult ITP Registry with respect to the general adult population in the UK.

Methods

Study Design

Data from the UK Adult ITP Registry and population-based studies in the medical literature were used to conduct a retrospective cohort study on the relative risk of TEs between adult patients with primary ITP and the general adult population.

Primary ITP Cohort

The disease cohort comprised adults (age > 16 years) with primary ITP attending an outpatient haematology clinic at Barts and The London NHS Trust between January 2000 and May 2005, at one of 10 collaborating UK centres between August 2002 and May 2005, or at one of 9 collaborating UK centres between October 2008 and March 2010 (Figure 2-5). Diagnoses were made in accordance with BCSH guidelines (*i.e.*, thrombocytopenia [*i.e.*, $< 150 \times 10^9$/L] in the absence of demonstrable causes such as HIV, HCV, or SLE).[2] Patients diagnosed as children but in whom primary ITP persisted into adulthood were eligible for inclusion.

Primary Outcomes and Covariates

The primary outcomes of interest were arterial, venous, and combined TEs, consisting of MI, UA, IS, and TIA; DVT and PE; and arterial and venous TEs, respectively. Covariates evaluated for the study included baseline (*i.e.*, at the time of diagnosis) participant characteristics (sex, age, referral status, laboratory results), ITP-specific treatments, and co-morbid conditions (Figure 2-1[a-d]). Data collection

was retrospectively performed via extraction of hospital medical records and, in the case of patients who died over the course of follow-up, examination of death certificates. Outcomes were considered prevalent if they had been recorded prior to the diagnosis of primary ITP or within 7 days thereof.

Follow-up and Censoring

Registry participants were retrospectively followed until censoring, death, or study closure in March 2010. Censoring took place at the time of discharge from clinic or first outcome occurrence. Thus, only first TEs were assessed.

Statistical Methods and Control Cohorts

IRs and 95% CIs of TEs were estimated assuming a Poisson distribution of events and were stratified by baseline age [dichotomous: ≥ 44 and < 44 years][*] and sex. Comparisons of event rates between patients within different strata of the primary ITP cohort were performed using Cox regression modelling. Assumptions of proportional hazards were assessed by fitting interaction terms between the stratifying variables and time, enabling evaluation of the change of HRs over time.

As the UK Adult ITP Registry consisted solely of patients with primary ITP, comparisons of TE IRs with disease-free adults were not possible using Registry data alone. Age and sex-stratified IR estimates of TEs were therefore drawn from population-based studies whose cohorts were sufficiently representative of the general adult population in the UK and used to calculate expected IRs within the primary ITP cohort (Tables 8-1 and 8-2). The ratios of observed-to-expected events served as estimates of the standardised rate ratios (SRRs) between the primary ITP cohort and the general adult population. Exact methods were used to calculate standard errors of the logarithms of the SRRs.

SRRs for MI, UA, IS, and TIA; for DVT and PE; and for MI, UA, IS, TIA, DVT, and PE were pooled using a DerSimonian and Laird random-effects meta-analyses to provide estimates for arterial, venous, and combined TE SSRs, respectively. All analyses were performed using Stata release 10 (StataCorp LP, College Station, Texas).

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[*] A pre-hoc decision was made to dichotomise the cohort by the median age of its members.

Ethics

The study was conducted under the auspices of the UK Adult ITP Registry, which has been approved for multi-center operation by the London Research Ethics Committee (Reference: 07/H0718/57) until mid-2017. Written informed consent was obtained from all patients prior to enrolment.

Table 8-1: Sex and Age-Stratified Incidence Rates of Arterial TEs from Population-Based Studies

Event	Author	Region *Country*	Study Period	Rates		
				Age Group *Years*	Males Incidence *Per 100,000 Person- Years*	Females Incidence *Per 100,000 Person-Years*
Myocardial Infarction (MI)	Goldacre[3]	Oxford *United Kingdom*	1994-1998	39 >	22	2
				40-44	48	9
				45-49	95	16
				50-54	159	27
				55-59	265	74
				60-64	425	151
				65-69	656	282
				70-74	915	456
				75-79	1,353	741
				80-84	1,812	1,091
				85 ≤	2,212	1,528
Unstable Angina (UA)	Rothwell *et al.*[4]	Oxford *United Kingdom*	2002-2005	35 >	1	0
				35-44	13	10
				45-54	60	18
				55-64	47	42
				65-74	97	123
				75-84	189	178
				85 ≤	397	70
Ischaemic Stroke (IS)				35 >	0	0
				35-44	35	21
				45-54	55	24
				55-64	187	119
				65-74	649	407
				75-84	913	982
				85 ≤	1,984	1,723
Transient Ischaemic Attack (TIA)				35 >	3	7
				35-44	13	5
				45-54	16	30
				55-64	74	105
				65-74	145	218
				75-84	327	561
				85 ≤	794	914

Table 8-2: Sex and Age-Stratified Incidence Rates of Venous TEs from Population-Based Studies

Event	Author	Region *Country*	Study Period	Rates		
				Age Group *Years*	Males	Females
					Incidence *Per 100,000 Years*	Incidence *Per 100,000 Years*
Pulmonary Embolism (PE)	Silverstein *et al.*[5]	Olmsted County Minnesota *USA*		0-14	0	0
				15-19	6	0
				20-24	12	5
				25-29	4	19
				30-34	16	0
				35-39	10	5
				40-44	42	11
				45-49	35	20
				50-54	51	58
				55-59	79	41
				60-64	50	90
				65-69	177	86
				70-74	363	154
				75-79	139	241
				80-84	656	546
				85 ≤	663	433
Deep Vein Thrombosis (DVT)				0-14	0	0
				15-19	6	11
				20-24	0	30
				25-29	13	31
				30-34	36	34
				35-39	34	19
				40-44	48	34
				45-49	49	41
				50-54	25	25
				55-59	69	91
				60-64	113	79
				65-69	163	136
				70-74	282	168
				75-79	307	193
				80-84	328	303
				85 ≤	120	396

Results

Baseline Characteristics and Prevalence of TEs

In total, 327 adults with primary ITP were retrospectively followed for a median time of 5.6 years (inter-quartile range: 2.4-9.2 years). Baseline characteristics of the primary ITP cohort are detailed in Table 2-1. Briefly, the mean age of patients was 42.9 ± 19.2 years, and a female-to-male ratio of 1.7:0 was observed. Of patients for whom data were available, 41.5% had been referred to their centre by a haematologist, and 81.5% were Caucasian (self-reported). The median baseline platelet count was 31 x 10^9/L (inter-quartile range: 9-80 x 10^9/L).

Table 8-3 reveals the baseline prevalence of TEs and co-morbid diseases within the primary ITP cohort. Nineteen patients (5.8%; 95% CI, 3.5-8.9%) had experienced a prior TE, with 16 (4.9%; 2.8%-7.8%) having suffered an arterial TE and 4 (1.2%; 0.3%-3.1%) a venous TE.[†]

[†] One patient experienced both arterial and venous TEs prior to diagnosis.

Table 8-3: Baseline Prevalence of TEs and Co-Morbidities

Event	Number of Participants	Percentage (95% CI)
TEs		
Combined TEs	**19**	**5.8 (3.5-8.9)**
Arterial TEs	**16**	**4.9 (2.8-7.8)**
Myocardial infarction (MI)	6	1.8 (0.7-4.0)
Unstable angina (UA)	6	1.8 (0.7-4.0)
Ischaemic stroke (IS)	5	1.5 (0.5-3.5)
Transient ischaemic attack (TIA)	4	1.2 (0.3-3.1)
Venous TEs	**4**	**1.2 (0.3-3.1)**
Deep vein thrombosis (DVT)	3	0.9 (0.2-2.7)
Pulmonary embolism (PE)	2	0.6 (0.1-2.2)
Co-Morbidities		
Hypertension	23	7.0 (4.5-10.4)
Autoimmune diseases	16	4.9 (2.8-7.8)
Autoimmune Haemolytic Anaemia	4	1.2 (0.3-3.1)
Reynaud's phenomenon	2	0.6 (0.1-2.2)
Rheumatoid arthritis	2	0.6 (0.1-2.2)
Autoimmune cytopenia	2	0.6 (0.1-2.2)
Eczema	2	0.6 (0.1-2.2)
Inflammatory bowel disease	1	0.3 (0-1.7)
Multiple Sclerosis	1	0.3 (0-1.7)
Psoriasis	1	0.3 (0-1.7)
Hypothyroidism	10	3.1 (1.5-5.6)
Renal disease	9	2.8 (1.3-5.2)
Miscarriage*	8	3.9 (1.7-7.5)
Osteoarthritis	8	2.4 (1.1-4.8)
Type 2 diabetes	8	2.4 (1.1-4.8)
Solid tumour	8	2.4 (1.1-4.8)
Hyperthyroidism	6	1.8 (0.7-4.0)
Candidiasis	4	1.2 (0.3-3.1)
Long-bone fracture	3	0.9 (0.2-2.7)
Cataracts	2	0.6 (0.1-2.2)
Liver disease	2	0.6 (0.1-2.2)
Haematological malignancy	2	0.6 (0.1-2.2)
Peptic ulcer disease	1	0.3 (0-1.7)

Incident TEs

Figure 8-1 presents a Kaplan-Meier curve of TE-free survival probabilities for the 308 adults with primary ITP who were free from vascular disease at baseline. Over a mean follow-up time of 7.2 ± 7.0 years, 16 incident TEs were recorded. The IR of combined TEs was 60.25 (95% CI, 34.99-103.77) per 10,000 patient-years (Table 8-4). Corresponding rates for arterial and venous TEs were 45.39 (95% CI, 24.42-84.36) and 17.81 (95% CI, 6.68-47.45), respectively. The most commonly experienced TE subgroups were MI: 22.06 (95% CI, 9.18-52.99) and IS: 22.09 (95% CI, 9.19-53.06). As expected, the IRs of arterial TEs were higher in males and the elderly (Table 8-5).

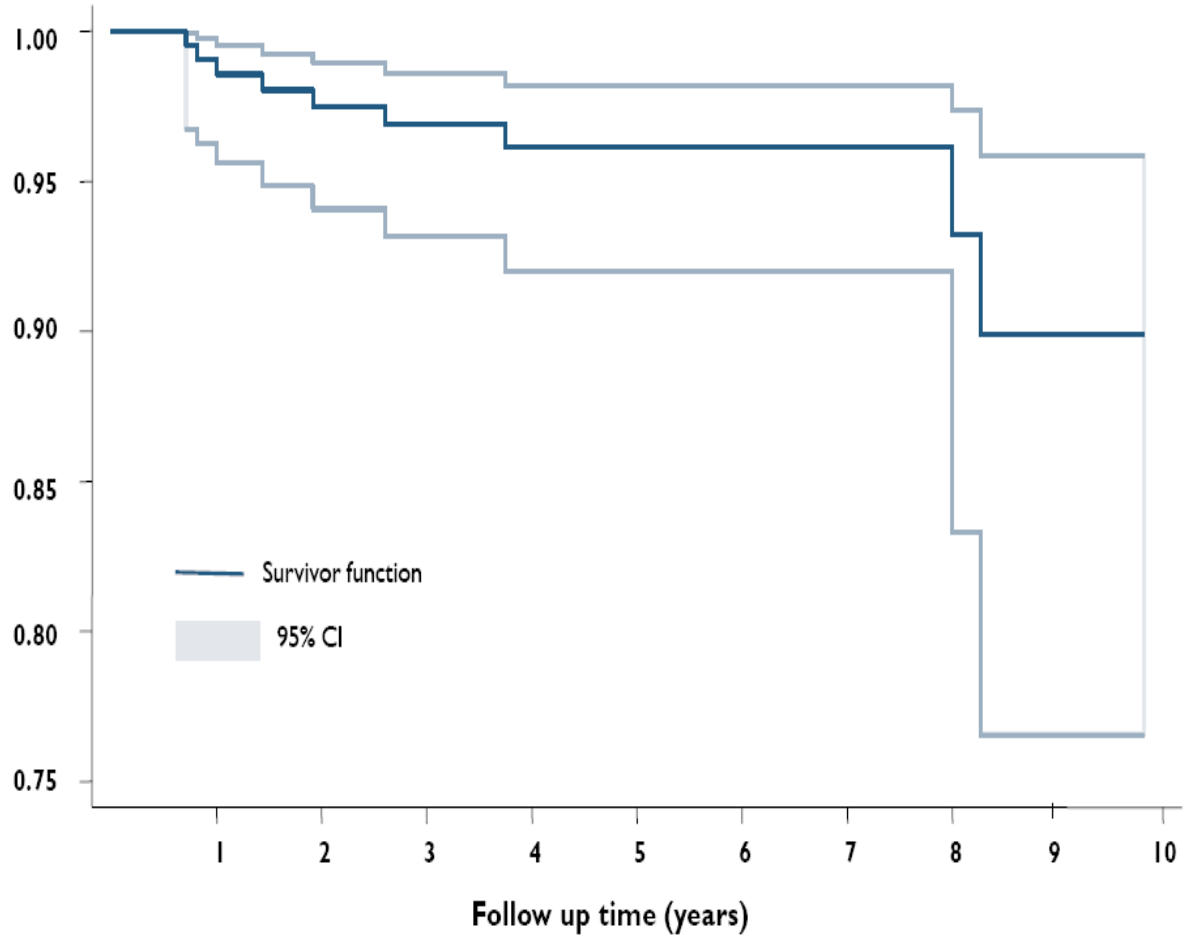

Figure 8-1: Kaplan-Meier TE-free survival estimate for 308 patients with primary ITP. 13 first incident TEs occurred during a mean follow-up time of 7.2 ± 7.0 years. Note: 19 (5.8%) patients from the UK Adult ITP Registry were excluded from the above analysis owing to existing vascular disease at baseline.

Table 8-4: Incident TEs among Registry Participants

Outcome	Person-Time *Patient-Years*	First Events‡	Incidence Rate (95% CI) *Per 10,000 Patient-Years*
Combined TEs	**2157.5**	**13**	**60.25 (34.99-103.77)**
Arterial TEs	**2203.1**	**10**	**45.39 (24.42-84.36)**
Myocardial Infarction (MI)	2266.8	5	22.06 (9.18-52.99)
Unstable Angina (UA)	2270.4	3	13.21 (4.26-40.97)
Ischaemic stroke (IS)	2263.9	5	22.09 (9.19-53.06)
Transient Ischaemic Attack (TIA)	2270.7	2	8.81 (2.20-35.22)
Venous TEs	**2246.0**	**4**	**17.81 (6.68-47.45)**
Deep Vein Thrombosis (DVT)	2252.8	3	13.32 (4.30-41.29)
Pulmonary Embolism (PE)	2278.5	2	8.78 (2.20-35.10)

Table 8-5: Incidence Rates and Hazard Ratios of TEs

Outcome	Stratum	Incidence Rate (95% CI) *Per 10,000 Patient-Years*	Unadjusted Hazard Ratio (95% CI)	Age- or Sex-Adjusted Hazard Ratio (95% CI)
Venous TEs	< 44 years	31.24 (11.72-83.23)		
	≥ 44 years	102.63 (53.40-197.24)	3.25 (0.99-10.68)	3.18 (0.97-10.44)
Arterial TEs	< 44 years	15.34 (3.84-61.32)		
	≥ 44 years	88.99 (44.50-177.95)	5.79 (1.20-27.86)	5.54 (1.16-26.42)
Venous TEs	Female	46.90 (22.36-98.37)		
	Male	90.24 (40.54-200.87)	1.87 (0.63-5.57)	1.87 (0.62-5.61)
Arterial TEs	Female	26.00 (9.76-69.28)		
	Male	90.24 (40.54-200.87)	3.32 (0.93-11.78)	3.11 (0.86-11.25)

‡ As patients were censored on the occurrence of a first event within each of the outcome categories shown, the total number of first arterial and first venous TEs does not correspond to the total number of first combined TEs (*e.g.*, a patients who experienced an MI followed by a DVT would have already been censored from the combined TE category at the time of his subsequent DVT. Similarly, the total numbers of first arterial and venous subgroup events do not correspond to the total numbers of first arterial and first venous TEs, respectively.

Standardised Rate Ratios

As detailed in Figure 8-2, the observed numbers of events for each of the specific TE outcomes were higher than those expected. Random effects meta-analyses yielded significant, pooled SRRs for combined (2.44 [95% CI, 1.58-3.79]), arterial (2.45 [95% CI, 1.48-4.06]), and venous TEs (2.43 [95% CI, 1.01-5.83].

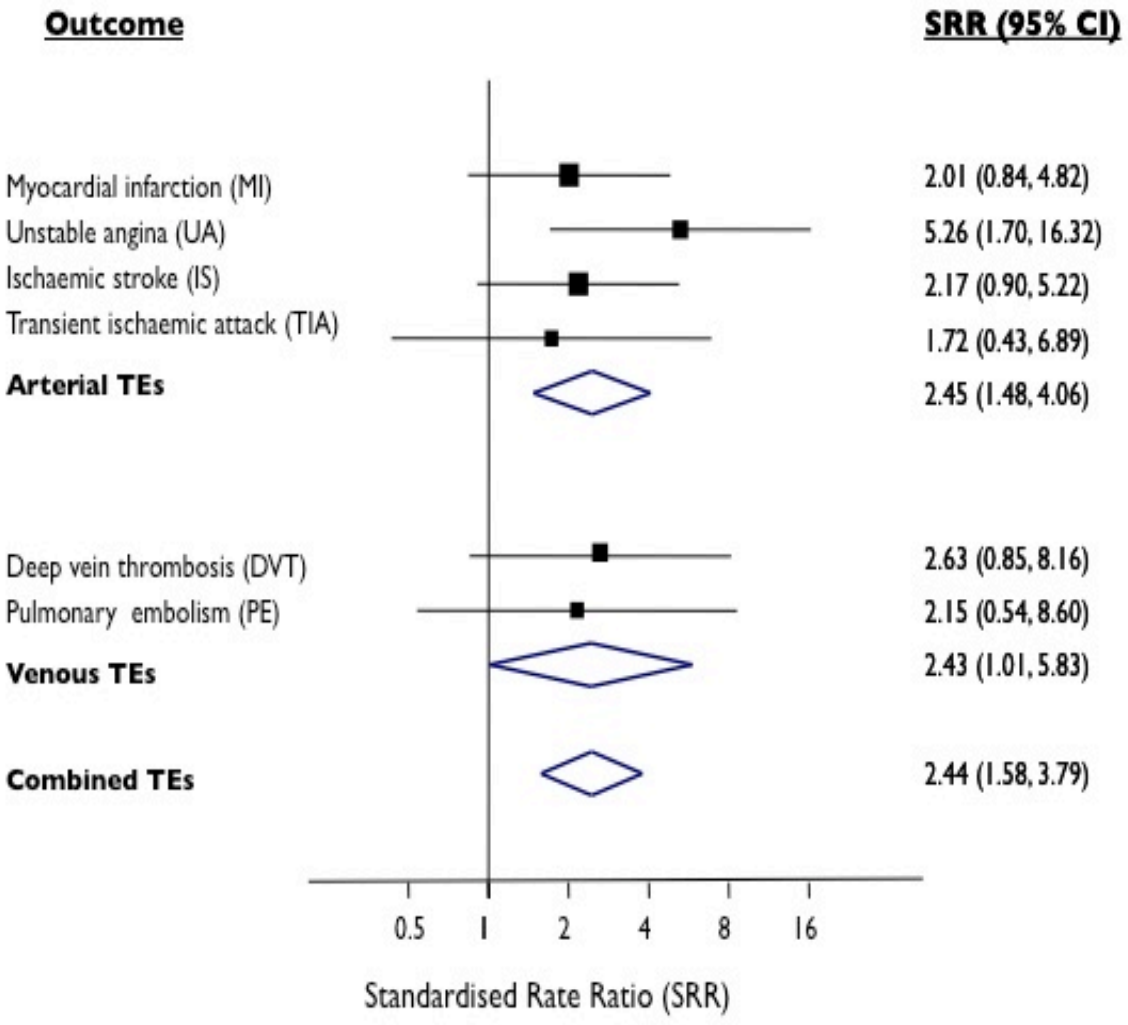

Figure 8-2: Standardised rate ratios (SRRs) of TEs in adult patients with primary ITP relative to the general adult population. MI, UA, IS, and TIA; DVT and PE; and MI, UA, IS, TIA, DVT, and PE SRR estimates were pooled using DerSimonian and Laird random-effects meta-analyses to calculate arterial, venous and combined TE SSRs, respectively. Significant heterogeneity across the outcomes was not observed (I^2 = 0.0%, p = 0.803).

Discussion

Results from this Registry-based study revealed over two-fold increased risks of arterial (SRR: 2.45 [95% CI, 1.48-4.06]), venous (SRR: 2.43 [95% CI, 1.01-5.83]), and combined (SRR: 2.44 [95% CI, 1.58-3.79) TEs among actively managed adults with primary ITP respective to the general adult population. SRR point estimates for each TE subgroup were, moreover, greater than one (Figure 8-2), demonstrating a consistency of effect. These findings strengthen observations made using the GPRD (Chapter 7)[6] and, importantly, suggest that increased risks of TE may not be restricted to patients with asymptomatic or only mildly symptomatic disease but likely extend to patients under continued follow-up for primary ITP.

Two primary limitations of the study should be noted. First, the absence of a disease-free cohort within the UK Adult ITP Registry prompted data usage from population-based studies for comparisons of TE IRs. As a result, study-level differences aside from primary ITP status may have contributed in part to observed differences in event rates (*i.e.*, ecological fallacy). Attempts were made to reduce the effect of such biases by selecting comparator cohorts representative of the source population of the primary ITP cohort.[§] The consistency of SRR estimates across TE subgroups suggested that impact of such biases were likely not appreciable.

Second, although data were collected on potential confounders such as administered treatments and co-morbid conditions, the size of this cohort was not sufficient to yield robust IR estimates within these strata. This inadequacy of statistical power reflects a general limitation of current studies into primary ITP and will be discussed at greater length in Chapter 9.

[§] For incidence rates of PE and DVT in the general adult population, the most comprehensive population-based study available was selected (Silverstein *et al.*). Although this study was set in the USA, it was felt that differences between its source population and that of the UK Adult ITP Registry were not appreciable.

Considerable work is required to evaluate whether the uncovered associations are representative of a causal relationship between primary ITP pathogenesis and TE onset. Although plausible mechanisms have been postulated, including a greater proportion of young, activated platelets in circulation and the increased thrombogenecity of platelet microparticles released following immune-mediated destruction,[7,8] it is similarly possible that the observed associations were due to confounding. As discussed in Chapter 7, for example, increased hospitalisation may have contributed to the observed association between primary ITP and venous TEs. Similarly, a high prevalence of antiphospholipid antibodies (*e.g.*, anticardiolipin and lupus anticoagulant antibodies) has been reported in adults with primary ITP.[9] These antibodies are hypothesised to trigger increased platelet activation and decreased production of prostacylcin, nitric oxide, and protein C,[10] which may additionally resulted in elevated IRs of TE subgroups.[10]

In conclusion, the present Registry-based retrospective cohort study of adults with primary ITP patients uncovered increased risks of arterial, venous, and combined TEs compared to the general adult population after adjustment for age and sex. These findings warrant further investigation in a larger cohort capable of direct within-study comparisons and adjustment for wider range of covariates.

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Chapter 9: Discussion

Summary

The results of this thesis implicate primary ITP as a pro-thrombotic condition in adults and provide evidence of a genetic contribution to the Th1 polarisation characterising the disease. Analyses conducted herein further yielded data demonstrating the utility of autologous ^{111}In-labelled platelet sequestration studies as an adjunct predictive tool prior to splenectomy and endorsing the routine testing for and eradication of *H. pylori* infection in patients residing in countries with a high prevalence of infection. Efforts are currently underway to develop a comprehensive, prospective international registry, which will ensure the availability of adequate sample sizes and, thus, statistical power for promising future research, including genome-wide association studies (GWAS) and investigations into the effects of the eradication of strain-specific *H. pylori* infection on platelet counts, the precise relative risk of non-response to splenectomy in patients with mixed or hepatic platelet sequestration, and the association of TEs with primary ITP in antiphospholipid antibody-negative adults with respect to the general adult population. Perhaps most importantly, these data may one day enable epidemiologists to construct, test, and validate prognostic models of disease trajectory in primary ITP so that intensive, potentially toxic treatments may be spared patients not at heightened risk of major haemorrhage.

Introduction

To further current understanding of disease pathogenesis, treatment effectiveness, and co-morbid burden among adults with primary ITP, this thesis aimed to characterise associations of primary ITP with both candidate SNPs and TEs, to assess the utility of autologous ^{111}In-labelled platelet sequestration studies prior to splenectomy, and to gauge the effectiveness of eradication therapy in elevating platelet count in *H. pylori*-infected patients. This chapter details the principal findings of these investigations and discusses their potential implications, strengths, and weaknesses, concluding with a summary of ongoing work and recommendations for future studies.

Data Sources

Data used for the aforementioned investigations were drawn from peer-reviewed published studies, the WTCCC 1958 British Birth Cohort, the GPRD, and the UK Adult ITP Registry. The development, initiation, and expansion of the latter database constituted an integral part of my doctoral studies. Over this time, comprehensive clinical data pertaining to co-morbid conditions (N = 25), treatments (N = 22), bleeding events, platelet counts, and other laboratory results (Figure 2-1) were successfully collected on 327 adults with primary ITP across 17 centres (Figure 2-5), spanning a median post-diagnosis period of 5.6 years (inter-quartile range: 2.4-9.2 years). DNA was further procured from 93 (44.9%) Caucasian participants comprising this cohort.*

Principal Findings

Candidate SNP Associations among Caucasians

Logistic regression modelling of associations between primary ITP and 6 candidate SNPs in cytokine or cytokine receptor genes in 268 Caucasian patients sex-matched (1:3) with healthy controls from the WTCCC 1958 British Birth Cohort showed a significant per rare allele OR of 1.34 (95% CI, 1.03-1.75) for *TNFA* -308 g>a (rs1800629). However, no significant associations were observed between these SNPs and either disease severity or *H. pylori* infection status.

The Utility of Autologous ^{111}In-labelled Platelet Sequestration Studies Prior to Splenectomy

Results from autologous ^{111}In-labelled platelet sequestration studies in 265 patients with primary ITP demonstrated a comparable median MPLS among patients with purely or predominantly splenic (25.9 hours [range: 6.8-407.0 hours]) and mixed or hepatic sequestration (31.1 hours [range: 7.2-271.0 hours]; p = 0.491). As expected in this non-blinded study, a highly significant difference was noted between the proportions of patients with purely or predominantly splenic (53.5%, N = 71) and mixed or hepatic sequestration proceeding to splenectomy (17.9, N = 20; p < 0.001).

Multivariable (gender, age at splenectomy, and MPLS) logistic regression analyses revealed increased odds of short (7.47 [95% CI, 1.89-29.43]), medium (4.85 [95% CI, 1.04-22.54]), and long-term (5.39 [95% CI, 1.34-21.65]) responses (platelet count > 100 × 10^9/L) to splenectomy in patients with purely or predominantly splenic sequestration and further highlighted an independent, inverse association between age at splenectomy and the likelihood of long-term success (0.95 [95% CI, 0.91-0.99]). Inclusion of post-ITP duration at the time of splenectomy as an additional covariate in subgroup analyses did not alter the significance of these results.

While illustrating excellent outcomes from splenectomy in patients with purely or predominantly splenic sequestration (median platelet count: 292 × 10^9/L [range: 14-589 × 10^9/L], number on treatment = 3 [4.3%]), long-term follow-up showed a similar proportion of patients reliant on treatment among the mixed or hepatic, splenectomised and the comparative, non-splenectomised cohorts (number on treatment: 6 [30.0%] vs. 28 [41.2%], respectively; p = 0.47).

Effects of the Eradication of H. pylori Infection on Platelet Count

A random-effects meta-analysis of 25 studies revealed a weighted, mean response (platelet count > 30 × 10^9/L and at least a doubling of the baseline count) to eradication therapy in 50.3% (95% CI, 41.6%-59.0%) of 696 evaluable, *H. pylori*-infected patients with primary ITP. Considerable heterogeneity was, however, observed between individual study findings. Exploration of these differences showed a direct correlation (r = 0.351, p = 0.018) between reported response and prevalence of infection among the source population.

Health-related lifestyle

Although children were more likely than adults to experience frustration over activity restrictions (23.3% vs. 9.5%, respectively; $p < 0.001$), the impact of primary ITP with regard to healthcare, insurance coverage, and social engagement was more pronounced in adults; 31.3% of adult patients reported having surgery delayed due to a low platelet count, and 30.2% experienced difficulty obtaining travel insurance. Notably, 12.5% of all patients surveyed reported "always" or "often" missing work or school due to fatigue.

TEs among Adults in the GPRD, the UK Adult ITP Registry, and Population-Based Studies

In a GPRD-based study, multivariable (ITP treatment and co-morbid conditions) Cox proportional hazards models revealed significantly increased risks of venous (HR = 1.58 [95% CI, 1.01-2.48]) and combined (HR = 1.41 [95% CI, 1.04-1.91]) TEs in 1,070 adults with primary ITP matched (1:4) with ITP-free patients on the basis of age, sex, primary care practice, and pre-diagnosis observation time. With the exception of PVT, which did not occur in the study population over follow-up (median time: 47.6 months [range: 3.0-192.5 months]), IRs of each venous TE subgroup (*i.e.*, PE, DVT, and other venous TEs) were elevated in the primary ITP cohort (Figure 7-1). Though an increased risk of arterial TEs was similarly observed (HR = 1.37 [95% CI, 0.94-2.00]), it did not achieve statistical significance. The above associations did not appear tied to the category of code used to identify patients with primary ITP, as higher IRs for venous and arterial TEs were seen with respect to the ITP-free cohort across both the idiopathic and autoimmune coding strata (Table 7-2).

A similar investigation, drawing data from the UK Adult ITP Registry and population-based studies with similar source populations, found increased, though non-significant, age and sex-adjusted risks of MI, UA, IS, TIA, DVT, and PE among patients with primary ITP relative to the general adult population (Figure 8-2). The pooling of these estimates via random-effects meta-analyses yielded significant SRRs for venous (2.43 [95% CI, 1.01-5.83]), arterial (2.45 [95% CI, 1.48-4.06]), and combined TEs (2.44 [95% CI, 1.58-3.79]).

Potential Implications

Disease Pathogenesis

The observed association between primary ITP and *TNFA* -308 g>a implicates a moderately increased disease susceptibility among carriers of the rare allele in the Caucasian adult population. Although this finding may represent a false positive result, there arguably existed a high prior probability of the association being true. Multiple studies have reported Th1 polarisation in primary ITP,[1-3] and *TNFA* -308 a has been tied to increased serum levels of TNF-α,[4] a cytokine that is critical for the development of Th1 responses.[5] However, the metaphorical littering of the literature with reports subsequently un-replicated genetic associations[6,7] underscores the need for cautious interpretation of this finding pending the results of adequately powered follow-up studies.

A validated association within a small series of candidate SNPs would suggest a high probability of uncovering further associations through GWAS, which have identified common susceptibility loci for such autoimmune diseases as ankylosing spondylitis, autoimmune thyroid disease, and multiple sclerosis.[8] It is nonetheless unlikely that genetic susceptibility is sufficient cause of primary ITP. As Ermann and Fathman[9] note, autoimmune disease concordance in monozygotic twins is less than 50%, highlighting the potential further requirement of an environmental trigger. The observed response to eradication therapy in 50.3% (95% CI, 31.8%-53.9%) of *H. pylori*-positive patients with primary ITP suggests that one such trigger may be *H. pylori* infection. This theory is bolstered by the fact that greater responses were reported in countries with a higher prevalence of infection. CagA-positive strains of *H. pylori* are similarly more common in these countries[10] and are capable of mimicking platelet antigens.[11,12] Semple *et al.*[13] and Bimczok *et al.*,[14] moreover, have recently reported enhanced Fc-dependent platelet phagocytosis in the presence of lipopolysaccharides from Gram-negative bacteria (*e.g.*, *H. pylori*) and Th1 induction by gastric dendritic cells in response to *H. pylori*, respectively. Collectively, these data highlight the potential role *H. pylori* infection in the onset of and exacerbation of primary ITP.

Treatment Effectiveness

The limited invasiveness of diagnostic tests, high observed response, and low toxicity profile and costs of treatment,[15] support the testing for and eradication of *H. pylori* infection in countries with a high prevalence of infection.

The finding of a significantly increased likelihood of response to splenectomy in patients with purely or predominantly splenic sequestration, meanwhile, highlights the utility of autologous ^{111}In-labelled platelet sequestration studies as an adjunct predictive tool prior to surgery. Although an insufficient number of patients with mixed or hepatic sequestration proceeded to splenectomy to enable precise estimation of the magnitude of this response, the observation of similar proportions of patients still reliant of therapy at last follow-up within the mixed or hepatic, splenectomised and comparative, non-splenectomised cohorts suggests that the adoption of alternate treatment strategies may have yielded comparable results for patients in the former cohort. However, considerable work is still needed to test this hypothesis.

Co-Morbid Disease Burden

Results from the health-related lifestyle survey highlight several possible avenues for patient advocacy and HRQoL research. Specifically, improved dialogue between patient support groups and insurers and between haematologist and surgeons would improve patient access to travel insurance and avoid unnecessary delays to elective surgeries, respectively.

The implications of associations of primary ITP with both venous and arterial TEs hinge on whether causal relationships were uncovered. To assess such a question, epidemiologists frequently employ the Bradford Hill criteria (Figure 9-1). A brief explanation of these criteria and evaluation of data follows.

I. *Strength:* Owing to the existence of multiple sources of potential bias in any investigation, a stronger observed association is more likely to reflect true associations and, thus, causation. Results from both the GPRD and UK Adult ITP Registry studies were generally strong, with those of the latter investigation revealing two-fold greater risks of venous and arterial TEs in patients with primary ITP relative to the general adult population.

II. *Consistency:* An association that has been noted by different research groups and in different settings is similarly more likely to be true. A significant association of primary ITP with venous TEs was seen in both studies. Moreover, the IRs of each venous TE subgroup were elevated in adults with primary ITP. A significant association of primary ITP with arterial TEs was, however, not found in the GPRD. Furthermore, the results of these studies have not yet been replicated by other groups.

III. *Specificity:* Causality may be more easily assigned to an isolated association. Paradoxically, the results of these studies implicate associations of primary ITP with both incident haemorrhages and TEs. Primary ITP has importantly also been tied to increased hospitalisation and antiphospholipid antibodies, themselves independent risk factors for TEs. In a study of 82 consecutive adults with primary ITP, for example, Diz-Küçükkaya *et al.*[16] reported a statistically significant difference in five-year, thrombosis-free survival between antiphospholipid-positive and negative patients in a study of 82 consecutive adults with primary ITP.

IV. *Temporality:* A cause must precede its effect. The requirement of temporality was met by both investigations, as analyses were restricted to first incident, post-diagnosis TEs.

V. *Biological Gradient:* More of a cause should lead to more of an effect. Though sparse, data from the GPRD study revealed elevated IRs of combined TEs in patients with more severe baseline thrombocytopenia (Figure 7-2), illustrating a potential dose-response effect.

VI. & VII. *Plausibility and Coherence:* A biological explanation that conforms to the existing knowledge base within a field provides credence to a proposed causal relationship. As discussed in Chapter 8, plausible mechanisms have been postulated for the onset of TEs in primary ITP, including a greater proportion of young, activated platelets in circulation and the increased thrombogenecity of platelet microparticles released following immune-mediated destruction.[7,8]

VIII. *Experiment:* If action can be taken to prevent a cause, its effect should abate. Long-term follow-up for patients in remission from primary ITP has not been conducted.

IX. *Analogy:* A causal argument would be strengthened were related effects observed to result in similar outcomes. Other thrombocytopenic autoimmune conditions, including idiopathic TTP[17] and SLE,[18] have been associated with TEs.

Although the associations of primary ITP with venous and arterial TEs both fulfil the majority of the Bradford Hill criteria, the above discussion highlights a more convincing case for causality with the former association, which exhibited greater consistency. However, there is still insufficient data to account for the role confounding may have played. In particular, it remains to be seen whether the associations persist in antiphospholipid antibody-negative patients. Were they to do so, discussions may be warranted on the utility of increased utilisation of thromboprophylactic treatment in patients at lower risk of haemorrhage.

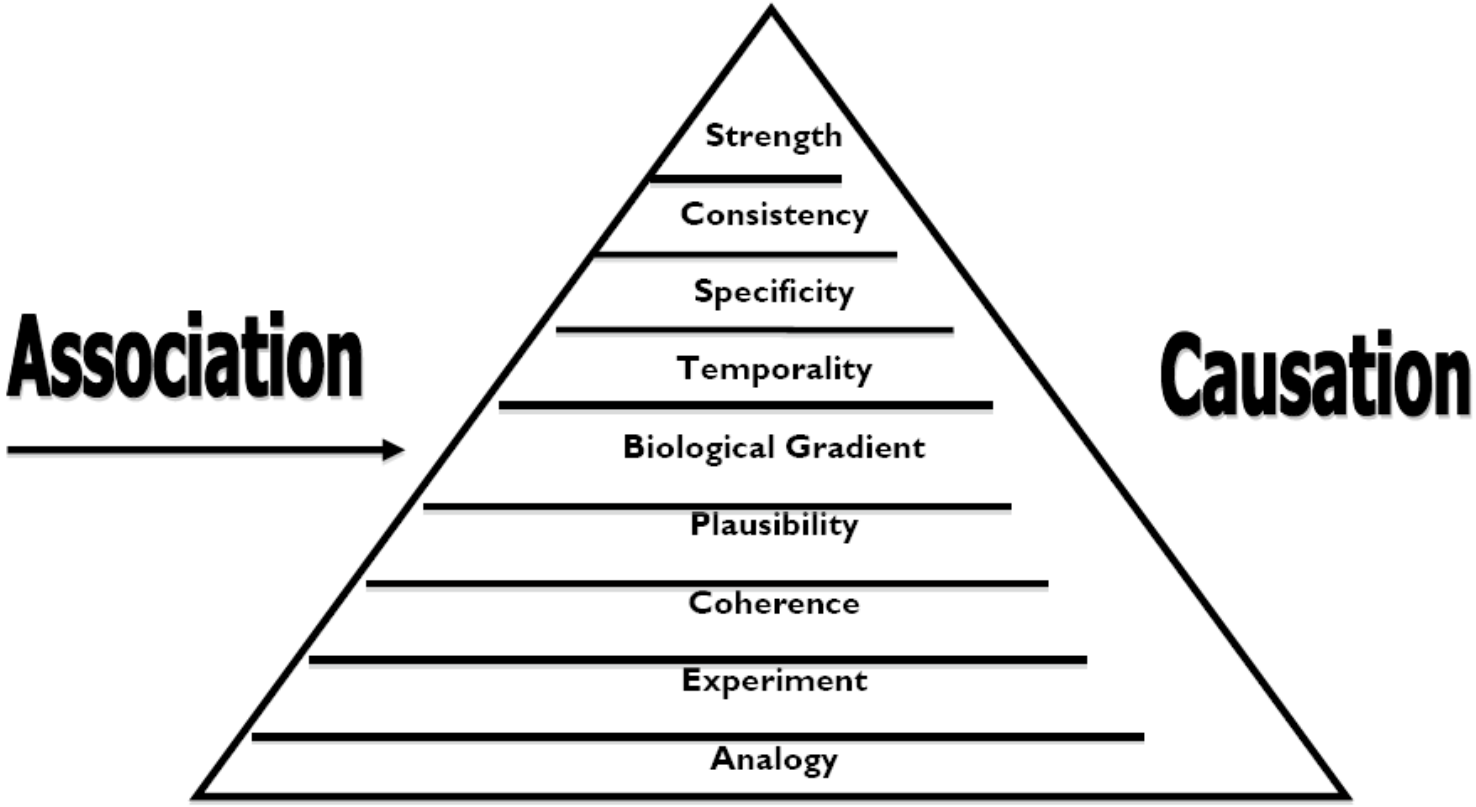

Figure 9-1: Bradford Hill criteria. In a lecture to the Royal Society of Medicine, Sir Austin Bradford Hill proposed using the 9 criteria in the analytic prism above to assess whether observed associations reflect causality. *Adapted from Bradford Hill A. The Environment and Disease: Association or Causation? Proceedings of the Royal Society of Medicine. 1956;58:295-300.*

Strengths and Limitations

The strengths of the UK Adult ITP Registry and of the investigations comprising this thesis warrant mentioning. Although still in its infancy, the Registry is already one of the largest repositories of clinical and biological data on adult patients with primary ITP in the world, and the external validity of its findings is likely high. The median post-diagnosis follow-up time captured by the Registry, 5.6 years (inter-quartile range: 2.4-9.2 years), was considerable, and, as enrolment was led by consultant haematologists, the likelihood of falsely classified participants within it was low. The studies presented herein have, meanwhile, successfully advanced current understanding of disease pathogenesis, treatment effectiveness, and co-morbid burden of adult ITP, having included the largest investigation of SNPs in primary ITP to date, arguably the most comprehensive review of the effects of eradication therapy on platelet count in *H. pylori*-infected patients, and the first investigations to compare the long-term outcomes from splenectomy and risks of TEs with comparative cohorts.†

Insufficient statistical power served as the primary limitation of this thesis, having hampered not only SNP and TE subgroup analyses, but also preventing precise estimation of the increased odds of response to splenectomy among patients with purely or predominantly splenic sequestration. This limitation was not surprising, for although among the most common haematological autoimmune diseases in adults, primary ITP is rare in absolute terms. As discussed in Chapter 1, the estimated 5-year period prevalence of the disease is 20.6-24.1 per 100,000 adults.[19,20] These figures underscore the importance of greater international collaboration among ITP specialists. Without such efforts, it is unlikely that suitable sample sizes will be available to investigate hypotheses generated from this work.

Two potential limitations additionally merit consideration. First, funding for the Registry was primarily provided by GSK, a pharmaceutical company with a newly licensed drug (eltrombopag) for primary ITP. It is therefore essential that the results of this thesis be viewed in light of the vested financial interests of this company. In particular, it can be argued that the conclusions reached from investigations into the utility of autologous ^{111}In-labelled platelet sequestration studies and risks of TEs in patients with primary ITP were of benefit to GSK, which may use these reports to position eltrombopag as an alternative to splenectomy in patients with mixed or hepatic sequestration and to defend against allegations that eltrombopag causes TEs, were they to occur. However, the analyses were designed independently by me, and the conclusions reached from the data generated were

mine alone. Despite these facts, studies have shown that the conclusions drawn by researchers may be influenced by the nature of the finding source.[21] In a systematic review of 370 RCTs, for example, Als-Nielsen *et al.*[22] found a five-fold greater odds of positive findings in cases where funding had been provided by a for-profit organisation. Therefore, the potential for such bias cannot be fully excluded.

Pharmaceutical funding of research is, however, a reality of the current academic medical system. In a 2007 study, Campbell *et al.*[23] reported that 60% of department chairs at American medical schools or teaching hospitals had personal relationships with industry. Evaluation of self-reported conflict of interest disclosures from the recently published international consensus guidelines for the management of primary ITP, moreover, revealed that 50% of clinician authors received research funding from or served as consultants for pharmaceutical companies with patented treatments for the disease.[24]

Second, selection bias may have been present in patient recruitment from external centres. As consecutive patients were not specifically targeted, it is possible that patients who were enrolled were systematically different from those were not. As discussed in Chapter 2, however, the mean age and platelet count did not differ significantly between participants from centres enrolling more than 3 patients and centres enrolling 3 or fewer patients (Table 2-3), suggesting that such bias was minimal.

Ongoing Work and Recommendations for Future Studies

Development of an International Adult ITP Registry

In November 2009, I proposed a decentralised model for an international adult ITP registry, in which lead regional centres would be recruited to develop and operate independent, affiliated regional registries. With the exception of a minimum number of required data fields, which would be shared with the international registry, lead regional investigators would have autonomy to tailor data and sample collection according to their interests and would further be able to apply for independent funding. In return for securing ethical approval to initiate their registry, directing regional recruitment, and performing routine validation of submitted data, the investigators would be offered a position on the advisory board of the international registry (Figure 9-2), which would wield decision-making power over alterations to the required data collection, anonymised data sharing with third parties (*i.e.,* pharmaceutical companies), and selection of studies for which the full repository of data may be used. This model will be piloted with the initiation of a Pan-American Adult

ITP Registry, which will be led by Dr. Provan, who will be joining the University of Toronto Faculty of Medicine as the Alexandra Professor of Benign Hematology in September 2010.

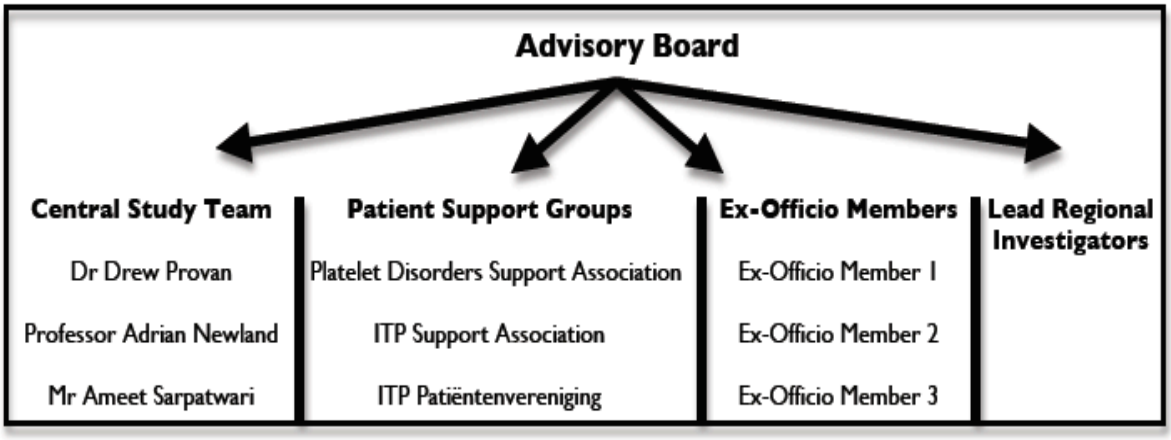

Figure 9-2: The structure of the advisory board for the proposed international adult ITP registry. Membership would be comprised of the central study team; the heads of the UK, America, and Dutch patients support groups; lead regional investigators, and three internationally recognised clinical experts on adult ITP (*i.e.*, ex-officio members).

Future Studies

Findings from this thesis support using data from the UK and Pan-American Adult ITP Registries to conduct GWAS and to investigate differences in both the response to eradication therapy in patients with CagA-positive and negative strains of *H. pylori* and the risk of TEs in antiphospholipid-negative patients with primary ITP and the general adult population. Studies should similarly be performed to estimate more precisely the increased risk of non-response to splenectomy in patients with mixed or hepatic sequestration and to evaluate whether more optimal predictive results are possible using alternate classification systems. As illustrated in Figure 9-3, the Najean *et al.*[25] scheme does not well categorise patients who exhibit extremely rapid and complete splenic pooling (*i.e.*, less than 30 minutes post-^{111}In-labelled platelet injection).

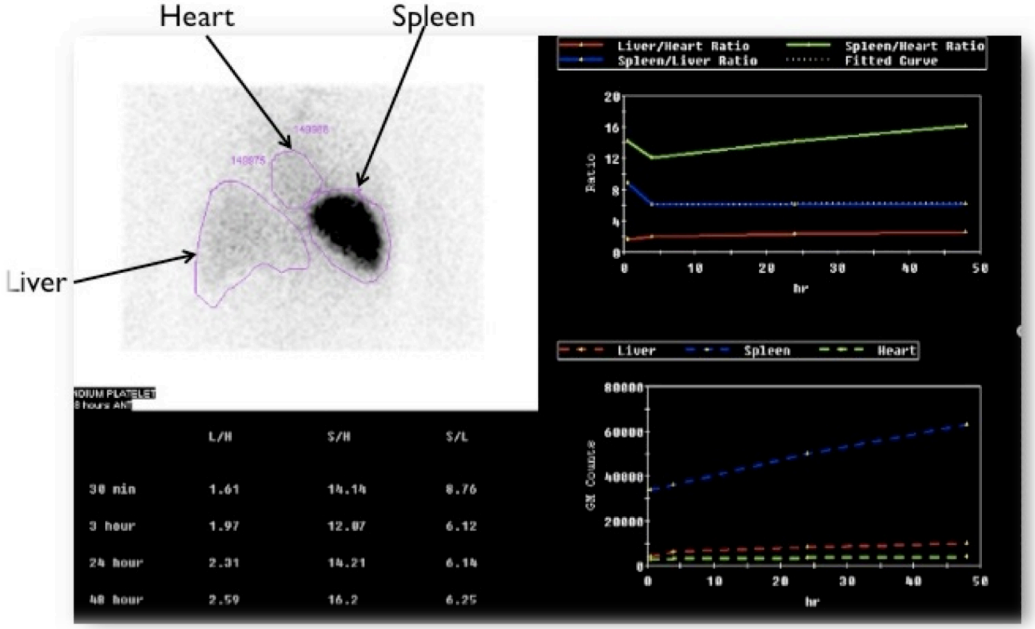

Figure 9-3: An autologous ^{111}In-labelled platelet sequestration study test result. Although sequestration appears centred in the spleen, this patient was categorised as having a hepatic pattern of platelet sequestration using the Najean *et al.* scheme.

Lastly, it is imperative that data from these Registries be used to improve current understanding of disease progression, a topic not directly investigated in this thesis. I am currently constructing an analysis plan to develop, test, and validate[26] a multivariable model of disease severity in adult patients with primary ITP (Figure 9-4). Considerable statistical power will be required to complete this project.

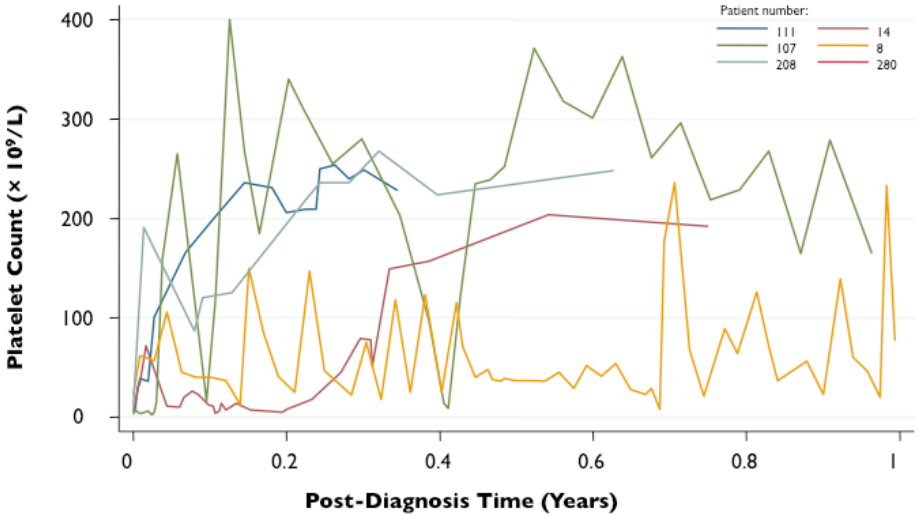

Figure 9-4: Examples of platelet count progression in adults with primary ITP. Disease trajectory will be modelled using baseline variables.

Conclusions

The results of this thesis support the concept of primary ITP as a pro-inflammatory (*i.e.*, TH-1 mediated), pro-thrombotic disease among adults. Analyses conducted herein reveal the utility of autologous ^{111}In-labelled platelet sequestration studies in identifying patients more likely to respond to splenectomy and strengthen calls for routine *H. pylori* testing and eradication in countries with a high prevalence of infection. Efforts to develop a comprehensive, prospective international registry will likely bear considerable fruit, ensuring the availability of adequate sample sizes and, thus, statistical power for promising future research, including GWAS of SNPs in primary ITP and investigations into the effects of the eradication of strain-specific *H. pylori* infection on platelet count, the precise relative risk of non-response to splenectomy in patients with mixed or hepatic platelet sequestration, and the association of TEs with primary ITP in antiphospholipid antibody-negative adults with respect to the general adult population.

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Appendix 1: Publications Authored

Journal Articles

Published or in Press

1.Stasi R, **Sarpatwari A**, Segal JB, et al. Effects of eradication of Helicobacter pylori infection in patients with immune thrombocytopenic purpura: a systematic review. Blood. 2009;113:1231-1240.

2.**Sarpatwari A**, Segal JB, Bussel JB, Stasi R, Osborn J. A case of rich fruit. Blood. 2009;113:6260.

3.**Sarpatwari A**, Bennett D, Logie JW, et al. Thromboembolic events among adult patients with primary immune thrombocytopenia (ITP) in the United Kingdom General Practice Research Database. Haematologica. 2010; 95:1167-1175.

4. **Sarpatwari A**, Watson S, Erqou S, et al. Heath-related lifestyle in adults and children with primary ITP. Br J Haematol. 2010. In Press.

5. **Sarpatwari A**, Provan D, Erqou, S, et al. Autologous ^{111}In-labelled platelet sequestration studies in patients with primary immune thrombocytopenia (ITP) prior to splenectomy: A report from the United Kingdom ITP Registry. Br J Haematol. 2010. In Press.

In Submission

6. Anzures-Cabrera J,* **Sarpatwari A**,* Higgins JPT.* Expressing meta-analyses of continuous outcomes in terms of risks. Stat Med. (* Joint First Authors)

7. **Sarpatwari A**, Bussel JB, Erqou S, et al. Single-nucleotide polymorphism analysis demonstrates a significant association of tumour necrosis factor-alpha (*TNFA*) with primary immune thrombocytopenia. Haematologica.

8. **Sarpatwari A**, Erqou S, Bennett D, et al. Thromboembolic events among adult patients with primary immune thrombocytopenia (ITP) in the United Kingdom Adult ITP Registry. Blood.

International Conference Posters

9. **Sarpatwari A**, Bennett D, Logie JW, et al. Thromboembolic events among adult patients with primary idiopathic thrombocytopenic purpura in the United Kingdom Adult ITP Registry. 50th ASH Annual Meeting and Exposition, San Francisco, CA, USA (December 2008). http://ash.confex.com/ash/2008/webprogram/Paper3059.html.

10. **Sarpatwari A**, Watson S, Anderson H, et al. Health-related lifestyle among adult and pediatric patients with idiopathic thrombocytopenic purpura in the United Kingdom. 50th ASH Annual Meeting and Exposition, San Francisco, CA, USA (December 2008). http://ash.confex.com/ash/2008/webprogram/Paper15633.html.

11. **Sarpatwari A**, Provan D, Sobnack R, et al. ^{111}In-labelled, autologous platelet sequestration studies in patients with primary immune thrombocytopenia (ITP): A report from the United Kingdom Registry. 51st ASH Annual Meeting and Exposition, New Orleans, LA, USA (December 2008). http://ash.confex.com/ash/2009/webprogram/Paper23999.html

Appendix 2: Talks and Patient Centred Activities

Talks at International Meetings

1. The United Kingdom Adult ITP Registry: Addressing unresolved epidemiological questions. **European ITP Support Group Meeting**, London, UK (June 2008).

2. The United Kingdom Adult ITP Registry: A framework for addressing unresolved epidemiological questions. **50th ASH Annual Meeting and Exposition**, San Francisco, CA, USA (December 2008).

3. The epidemiology of autoimmune diseases and role of registry studies. **European School of Haematology: Haematological Aspects of Autoimmune Diseases**, Mandelieu, France (February 2009). http://www.multiwebcast.com/esh/2009/AutoImmune/3012/ameet.sarpatwari.epidemiology.of.autoimmune.disease.&.role.of.registry.studies.html.

4. Autologous, ^{111}In-labeled platelet sequestration studies in patients with primary ITP: A report from the United Kingdom Adult ITP Registry. **51st ASH Annual Meeting and Exposition**, New Orleans, LA, USA (December 2009).

5. How useful are autologous, ^{111}In-labelled platelet sequestration studies in patients with primary ITP? Lessons from the United Kingdom Adult ITP Registry. **European School of Haematology: Immune Thrombocytopenia**, Lisbon, Portugal (April 2010). http://www.multiwebcast.com/esh/2010/ITP/speakers/72268/mr.ameet.sarpatwari.biography.html.

Talks at National Meetings

6. Disease progression, treatment effectiveness, and co-morbidities among adult patients with primary idiopathic thrombocytopenic purpura (ITP) in a United Kingdom Cohort. **ITP National Convention**, Oxford, UK (April 2007).

7. Analysis of the ITP Support Association lifestyle survey. **ITP National Convention**, London, UK (November 2008).

Patient Centred Activities

8. **Contributing writer**, *The Platelet*, the official newsletter of the ITP Support Association.

9. **Administrator**, the United Kingdom Adult ITP Registry Patient Forum, http://ukitpregistry.com/phpBB2/index.php.

10. **Co-host**, United Kingdom Adult ITP Registry Podcasts, http://www.ukitpregistry.com/UKITPEducation/UKITPPodcasts.html.

Appendix 3: The UK ITP Support Association Health-Related Lifestyle Survey

Please return this questionare to the address on the last page as soon as possible and not later than 31st October 2007.
Thank you.

If it is your child who has ITP please encourage them to complete the questionnaire on their own. If answering on behalf of a child then please try to answer as they would have done about their ITP.	**Details of person with ITP:** Name _____ Day Month Year Date of Birth ☐ ☐ ☐ Male Female Gender ☐ ☐ Duration of ITP ☐ Years or ☐ Months Most recent platelet count. ☐
Wording Never = none of the time Rarely = almost none of the time Sometimes = once in a while Often = almost all of the time Always = all of the time	
All responses to this questionnaire will be treated in the strictest confidence.	If you are completing this form on behalf of a child please state your relationship to the child: ☐

Please underline or circle the answers					
1. Do you tell your friends about your ITP?	Never	Rarely	Sometimes	Often	Always
2. How often do you have bruises?	Never	Rarely	Sometimes	Often	Always
3. How often do you have bleeding?	Never	Rarely	Sometimes	Often	Always
4. What type of bleeding or bruising have you had in the last month? Please **score** each between 0 (none) to 4 (very bad) or circle yes or no after "I am in remission".	Bruising ☐ Nose bleeds ☐ Mouth bleeds ☐ Blood in stool ☐		Blood in urine ☐ Heavy periods ☐ Other ☐ ☐ I am in remission : yes no		
5. Do you try to hide your bruises?	Never	Rarely	Sometimes	Often	Always
6. Does the bruising ever stop you from going out?	Never	Rarely	Sometimes	Often	Always
7. Does your ITP ever stop you going to work or school?	Never	Rarely	Sometimes	Often	Always
8. How many days have you missed in the last six months because of:-	Bleeding ☐ Bruising ☐ Hospital visits ☐		Drug side effects ☐ Other ☐ ☐		

9. Are people ever suspicious that the bruises are a result of physical violence?	Never	Rarely	Sometimes	Often	Always

10. If so, who has been suspicious or commented adversely about your bruises? Please underline or circle the answers.

Stranger Family Friends Teacher Doctor No-one Other ☐

Please provide examples of comments or further details at the end of questionnaire:

11. I get bothered because I can not do the activities I like.	Never	Rarely	Sometimes	Often	Always

12. What activities are you unable to do? Please underline or circle the answers.

Football Rugby Swimming Go out with friends Go on holiday Other ☐

13. If unable to do an activity, why? Please underline or circle the answers.

Low platelet count Too much bruising Fear of bleeding Other ☐

14. Where have you had advice about what activities you can do? Please underline or circle the answers.

Hospital Doctor GP ITP Support Association Internet Other ☐

15. I am concerned that I do not know enough about my ITP.	Never	Rarely	Sometimes	Often	Always
16. I was concerned that my hospital doctor did not know enough about my ITP.	Never	Rarely	Sometimes	Often	Always

Q17 to Q22. Which of the following have been helpful in providing information and help about ITP

17. My hospital doctor when the ITP was first diagnosed	Never	Rarely	Sometimes	Often	Always
18. GP	Never	Rarely	Sometimes	Often	Always
19. ITP Support Association	Never	Rarely	Sometimes	Often	Always
20. Internet	Never	Rarely	Sometimes	Often	Always
21. ITP Specialist	Never	Rarely	Sometimes	Often	Always
22. Other ☐	Never	Rarely	Sometimes	Often	Always

23. Referring only to Q17, my hospital doctor was an:-	Adult Haematologist Paediatric Haematologist Paediatrician	A&E doctor Adult non-haematologist Other ☐
24. My current ITP specialist is:-	Adult Haematologist Paediatric Haematologist Paediatrician	I have never seen a specialist Other ☐ Don't know

25. How often would you like to see an ITP specialist?	Never	At diagnosis only	After 6 months only	All the time

26. How far would you be prepared to travel to see a specialist? ☐ miles or ☐ hours

27. Have you ever been refused a referral to an ITP specialist or hospital of your choice?	Yes	No			
28. Do you have difficulty getting dental work done because of ITP?	Never	Rarely	Sometimes	Often	Always
29. Have you had surgery (other than splenectomy for ITP) postponed or delayed because of a low platelet count?	Yes	No			
30. Have you had difficulty obtaining or been refused insurance? Please underline or circle Travel insurance Life insurance Other ☐					
31. Have you been unable to have a tattoo or body piercing because of ITP?	Yes	No			
32. Are you currently receiving medication from your doctor for ITP	Yes	No			
33. Is your doctor giving you treatment because : Your symptoms are troublesome, Your platelet count is low, Don't know					
34. Have you ever taken prescribed drugs for your ITP.	Yes	No			
35. If yes, were you concerned about possible side effects of drug treatment?	Yes	No			
36. Have you ever tried alternative therapies or vitamin & mineral supplements for your ITP?	Yes	No			
37. If yes were you concerned about possible side effects of alternative therapies or supplements?	Yes	No			
38. Did the onset of your ITP appear to follow a viral infection (i.e. within 14 days)?	Yes	No			
39. If yes, which virus did you have? ☐					
40. Does your ITP have an impact on other members of your family?	Never	Rarely	Sometimes	Often	Always
41. Have you been bothered by tiredness or fatigue that you attribute to your ITP?	Never	Rarely	Sometimes	Often	Always
42. Have you ever been unable to go to work or school because of tiredness and fatigue?	Never	Rarely	Sometimes	Often	Always
43. Do you live in:- England, Scotland, Wales, Northern Ireland, Other ☐					

Signature _____ Date _____

Please return this questionnaire as soon as possible and not later than 31st October 2007 to the address on the next page. Thank you.

PTO

Appendix 4: Proposal Submitted to GSK for Registry Funding

Cover Sheet

Title:

Disease Progression, Treatment Effectiveness, and Co-Morbid Conditions among Adult Patients with Primary Immune Thrombocytopenia (ITP) in a United Kingdom Cohort

Abstract: (For Internal Use Only – limit to 20 lines max):

The efficacy of eltrombopag/promacta (SB-497115-GR) in elevating peripheral platelet counts among adult patients with primary immune thrombocytopenia (ITP) in clinical trials has helped fuel the drive to meet post regulatory needs, namely the timely collection of further epidemiological information on this poorly researched condition to assist safety-monitoring and marketing efforts.

As a potential complement to the ITP sample drawn from the General Practice Research Database (GPRD), the 734-participant, adult ITP cohort (UKITP Cohort) initiated by Dr. Drew Provan at the Royal London Hospital (RLH) and enrolees in the Revised Adult ITP Registry may help provide such data. Investigation into this combined study population via systematic extraction of patient-records presents a unique opportunity for uncovering information concerning the progression, treatment effectiveness, and co-morbid burden of adult ITP. Though resource-intensive, it is believed that such an undertaking will ultimately prove both feasible and fruitful, yielding findings applicable to the general and refractory populations of adult patients with primary ITP.

Authors:

Mr. Ameet Sarpatwari and Drs. Drew Provan, Dimitri Bennett, Jamie Robinson, and Sue Hall

Revision Chronology: Revision 2.1

Compound Numbers/Keywords (if applicable): Eltrombopag (Promacta)

Electronic File name and location: N/A

CONFIDENTIAL

GlaxoSmithKline Research and Development – Worldwide Epidemiology

Title Page

Title:

Disease Progression, Treatment Effectiveness, and Co-Morbid Conditions among Adult Patients with Primary Immune Thrombocytopenia (ITP) in a United Kingdom Cohort

Date of Protocol :

This Revision 2.1: 05 September 2010

Epidemiology Project/Protocol Number:

WEUSRTP1121

Principal investigator(s) and center(s) involved in the study (as appropriate):

Dr. Drew Provan
Room 417, Pathology and Pharmacy Building
80 Newark Street
The Royal London Hospital
London E1 2ES

Dates of observation:

Time of Diagnosis-December 2010

Database source (if appropriate):

Self-Assembled Database

CONFIDENTIAL

GlaxoSmithKline Research and Development – Worldwide Epidemiology

Approval Form

Product/Research Compound Name/TMT:

Eltrombopag (Promacta)

Epidemiology Study/Project #:

WEUSRTP1121

Study Title:

Disease Progression, Treatment Effectiveness, and Co-Morbid Conditions among Adult Patients with Primary Immune Thrombocytopenia (ITP) in a United Kingdom Cohort

Project Officer Name (Primary):Project Officer Name (Secondary):

Dr. Dimitri Bennett

Name(s) of Principal Investigator(s) or Data Source(s):

Dr. Drew Provan

Mr. Ameet Sarpatwari

List of Abbreviations

BCSH: British Committee for Standards in Haematology

DVT: Deep vein thrombosis

FDA: Food and Drug Administration

GPRD: General Practice Research Database

GSK: GlaxoSmithKline

HCV: Hepatitis-C

IS: Ischaemic stroke

ITP: Primary immune thrombocytopenia

IVIg: Intravenous immunoglobulin

MI: Myocardial infarction

NRES: National Research Ethics Service

NDA: New Drug Application

Non-RLH Sub-Cohort: Patients from the UKITP Cohort not registered at the RLH

PE: Pulmonary embolism

REC: Multi-Centre Research Ethics Committee

RLH: Royal London Hospital

RLH UKITP Sub-Cohort: Patients from the UKITP Cohort registered at the RLH

SNP: Single nucleotide polymorphism

TPO: Thrombopoietin

UK: United Kingdom

UKITP Cohort: The adult ITP cohort assembled by Dr. Drew Provan

WWEpi: Worldwide Epidemiology

TABLE OF CONTENTS

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1. SUMMARY OF PROTOCOL

The efficacy of eltrombopag/promacta (SB-497115-GR) in elevating peripheral platelet counts among adult patients with primary immune thrombocytopenia (ITP) in clinical trials has helped fuel the drive to meet post regulatory needs, namely the timely collection of further epidemiological information on this poorly researched condition to assist safety-monitoring and marketing efforts.

As a potential complement to the ITP sample drawn from the General Practice Research Database (GPRD), the 734-participant, adult ITP cohort (UKITP Cohort) initiated by Dr. Drew Provan at the Royal London Hospital (RLH) and enrolees in the Revised Adult ITP Registry may help provide such data. Investigation into this combined study population via systematic extraction of patient-records presents a unique opportunity for uncovering information concerning the progression, treatment effectiveness, and co-morbid burden of adult ITP. Though resource-intensive, it is believed that such an undertaking will ultimately prove both feasible and fruitful, yielding findings applicable to the general and refractory populations of adult patients with primary ITP.

2. INTRODUCTION

ITP is an autoimmune condition characterised by decreased levels of peripheral platelets (< 150 × 10^9/L) resulting from premature destruction of autoantibody-coated platelets by the reticuloendothelial system and suboptimal megakaryocytic platelet production.[1-3] ITP is a condition of imprecise aetiology and, as such, remains diagnosable only through thorough exclusionary clinical evaluation.[4] Primarily acute (< 6 months) in duration among children, ITP is believed to manifest itself predominantly chronically among adults, giving visible rise to bruising and petechiae of the skin and increased susceptibility to major bleeding events, including intracranial haemorrhage.[1]

Estimates of the incidence rate of ITP among adults range from 1.6-6.6 cases per 100,000 person-years.[1] However, epidemiological investigation into this condition has been sparse, throwing into sharp relief the uncertainty of these figures and the incompleteness of data with which to uncover disease progression, first-line treatment effectiveness, and both natural and treatment-induced co-morbid conditions.[5;6]

Since 2000, Dr. Drew Provan, an internationally recognised ITP expert and consultant haematologist at the RLH in Whitechapel, has worked to establish an adult ITP registry in the United Kingdom (UK). Before halting the project temporarily in 2005, he had successfully managed to identify 734 afflicted patients (UKITP Cohort), storing DNA samples (2x ~5mL) for over 40% of these individuals for future gene-association testing. Full reporting of all adult patients with primary ITP is, however, only known to have transpired at the RLH haematology clinic, which documented 332 consecutive patients from 2000 to 2005 (RLH UKITP Sub-Cohort).

As illustrated by Table 1, the female:male distribution of these patients overlaps well with the ratio believed to characterise the general population of adult patients with primary ITP. Estimates of median age of diagnosis among both genders vary considerably in the epidemiological literature, preventing the use of this trait for precise veridical assessment. Nevertheless, knowledge that ITP remains a hospital-based diagnosis in the UK and that the RLH serves as a high-volume primary haematological referral centre suggests that primarily referred participants within this sub-cohort are well representative of the general population of adult patients with primary ITP. Remaining RLH Sub-Cohort participants constitute patients secondarily referred from clinics throughout the UK. These individuals were referred almost exclusively as a result of first-line treatment failure and are, thus, considered potentially representative of the refractory, primary, adult ITP population. A comprehensive gauge of the external validity of these participants will only be possible following an analysis of referral patterns among consultant haematologists. Owing to the possible existence of a high referral threshold among specialists, these secondarily referred participants may ultimately prove more representative of the severe end of the refractory spectrum.

Table A4-1: Demographic Comparison of the RLH UKITP Cohort with Population & Hospital-Based Estimates

Demographic Variable	RLH UKITP Sub-Cohort Subset (N=109) Age at Diagnosis	Hospital-Based Adult ITP Cohort[7] Age at Diagnosis	Population-Based Adult ITP Cohort[8] Age at Diagnosis
Age	39.2 (mean); 35.4 (median)	N/A	56 (median)
Female:Male Ratio	1.7:1	1.7:1.0	1.7:1.0
Female Age	37.6 (mean); 34.9 (median)	37 (mean)	56 (median)
Male Age	41.7 (mean); 37.9 (median)	41 (mean)	62 (median)

More ambiguity exists concerning the external validity of the second sub-cohort, the remaining 402 adult patients with primary ITP submitted for enrolment by consultant haematologists throughout the UK from 2002 to 2005 (Non-RLH UKITP Sub-Cohort). Approximately one-fifth of consultant haematologists in the UK (76) enrolled at least one consenting adult patient with primary ITP for study inclusion. On average, 3.6 patients were submitted per consultant, suggesting the possible inclusion of only a subset of the overall patient population visiting these clinics. At present, it is unclear to what degree participants comprising the enrolled sample are representative of this larger population. However, their potential to unlock valuable information concerning disease progression, treatment effectiveness, and co-morbid disease burden in ITP is nonetheless strong.

Representatives from GlaxoSmithKline (GSK) have expressed interest in the data that may be uncovered from the UKITP Cohort and, further, from all participants enrolled during the first two years of the Revised Adult ITP Registry launched in October 2008. The company and one of its global competitors, Amgen, have both developed novel thrombopoietin (TPO) agonists, eltrombopag/promacta and AMG 531/romiplostin respectively, which have shown favourable phase I, II, and III results in safely elevating platelet counts among adult patients afflicted with chronic primary ITP.[9;10] However, owing to limited epidemiological research into this condition, there exists a paucity of descriptive and analytical information from which to base safety and data monitoring efforts, treatment indications, and marketing strategies. There has, therefore, been a convergence of clinical, regulatory, and epidemiological support for investigating accessible ITP populations.

Investigation of patients' hospital medical records suggested that information could be validly obtained through the development and implementation of a systematic extraction, a resource-intensive and, therefore, costly undertaking. Having carefully weighed the merits and potential drawbacks of initiating the extraction process, we have reached the conclusion that such expenditure would ultimately prove both feasible and worthwhile.

3. OBJECTIVES

a) To compile an anonymised database of demographic, ITP-specific, and co-morbid disease information on UK patients representative of adult patients with primary ITP

b) To describe and categorise disease progression of adult patients with primary ITP via platelet count and bleeding events

c) To document treatment practices for adult patients with primary ITP

d) To estimate the burden of co-morbid conditions among adult patients with primary ITP

   i. To estimate the prevalence and incidence of cataracts, osteoarthritis, type II diabetes, hypertension, peptic ulcers, *H. pylori* infection, renal failure/impairment, chronic liver disease, myocardial infarction (MI), ischaemic stroke (IS), transient ischaemic attack (TIA), unstable angina (UA), deep vein thrombosis (DVT), pulmonary embolism (PE), splenomegaly, anaemia, thyroid disease, depression/anxiety, miscarriage, Cushing's syndrome, *Candida* infection, pneumonia, other autoimmune disease, haematological malignancy, solid tumour malignancy, phototoxicity, and mortality (incidence only) among the primary adult ITP populations at the time of and following both diagnosis and treatment

e) To develop and test a prognostic model for major bleeding among adult patients with primary ITP

f) To test the relationship of thromboembolic events with (i) antiphospholipids at presentation, (ii), co-morbid conditions, (iii) common ITP treatments, and (iv) platelet count-categories at presentation among adult patients with primary ITP

g) To test the relationship between (a) autologous ^{111}In-labelled platelet sequestration pattern and (b) complete response at (i) 1-3 months, (ii) 6-12 months and (iii) last follow-up post splenectomy in adult patients with primary ITP

h) To launch an international registry of adult patients with primary ITP

4. RATIONALE OR CONTRIBUTION TO GSK

Eltrombopag/Promacta (SB-497115-GR), a GSK-patented TPO agonist, has shown favourable Phase II and III results in significantly boosting peripheral platelet counts among adult ITP patients, prompting New Drug Application (NDA) filing with regulatory agencies in both the United States and Europe.

However, as previously detailed, there exists at present limited epidemiological information concerning disease progression, treatment effectiveness, and both natural and drug-induced co-morbidities of ITP among adults:

> *Various clinical dilemmas persist in newly diagnosed ITP…These include an estimation of the bleeding risk and the need for treatment in the individual patient and the inability to predict the disease course for the individual patient at the time of diagnosis.*[11]

Such information will be crucial for monitoring and interpreting long-term safety signals, for establishing evidence-based treatment guidelines, and for designing efficient marketing strategies. Analyses conducted on the information collected from this cohort will help provide these details. In this respect, the reports submitted as part of this initiative may be used to supplement the Safety Database submitted to the Food and Drug Administration (FDA) and other regulatory agencies.

5. TARGET AUDIENCE

Members of the ITP team at GSK have been fully supportive of this study, having expressed particular interest in obtaining detailed information concerning the disease progression in ITP via longitudinal peripheral platelet count and bleeding events as well as prevalence and incidence estimates of natural and drug-induced co-morbid conditions. As aforementioned, it is possible that the reports submitted as part of this study will be reviewed by regulatory agencies. These reports may further be placed on the Clinical Trial Registry at the discretion of the Worldwide Epidemiology (WWEpi) oncology division at GSK.

6. METHODOLOGY

6.1 Study Population

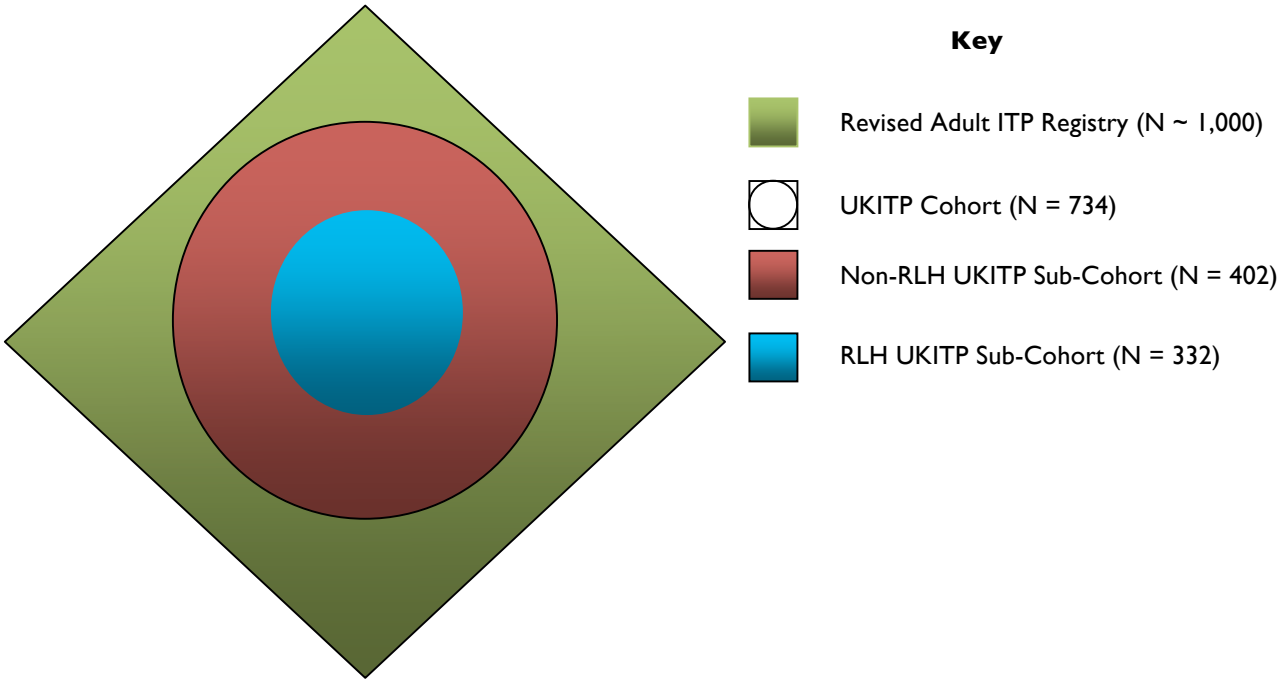
The study population will be comprised by the existing 734-participant, adult ITP cohort (UKITP Cohort) initiated by Dr. Drew Provan at the Royal London Hospital (RLH) as well as all enrolees in the Revised Adult ITP Registry launched in October 2008.

The UKITP Cohort may itself be broken down into two distinct sub-cohorts; the first of these groups constitutes all adult patients with primary ITP having visited the RLH haematology clinic from 200 to 2005 (RLH UKITP Sub-Cohort). As previously documented, the female:male ratio of these 332 patients (1.7:1) is in accordance with population-based estimates of the sex distribution among the primary adult ITP population.[8;12] Although conclusions concerning the external validity of this sample are difficult to extrapolate, assumptions that primarily and secondarily referred patients comprising it well represent the general and refractory populations of adult patients with primary ITP, respectively, appears sound; ITP is hospital-diagnosed in the United Kingdom, and the RLH UKITP Sub-Cohort represents a full sampling of a high-volume primary and secondary referral centre.

Admittedly, more ambiguity exists concerning the external validity of the second sub-cohort, the remaining 402 adult patients with primary ITP submitted for enrolment by consultant haematologists throughout the UK from 2002 to 2005 (Non-RLH UKITP Sub-Cohort). Approximately one-fifth of consultant haematologists in the UK (76) enrolled at least one consenting, adult patient with primary ITP for study inclusion. On average, 3.6 patients were submitted per consultant, suggesting the possible inclusion of only a subset of the overall patient population visiting these clinics. At present, it is unclear to what degree participants comprising the enrolled sample are representative of this larger population. However, owing to the relative rarity of ITP in the general adult population, the paucity of available epidemiological data on this condition, and the roughly commensurate investment of resources required for a cursory versus comprehensive investigation, it was deemed prudent to proceed with a full extraction of the hospital medical records of these patients.

Investigation into the UKITP Cohort will be complemented by the Revised Adult ITP Registry, which is open to all residents in the UK. As illustrated by Figure 1, the Revised Adult ITP Registry = automatically subsumed all patients in the UKITP Cohort upon its initiation in October 2008. In addition to undergoing a retrospective evaluation upon enrolment, all Revised Registry participants will be prospectively followed at annual intervals. The final component of the study population, then, will consist of all patients enrolled during the first two years of the Revised Registry.

Figure A4-1: Study Population



The validity of identified primary ITP cases represents a key strength of this study. Enrolled participants have all been explicitly diagnosed and submitted for study inclusion by specialists, arguably the gold-standard for case ascertainment for exclusionary conditions. Due to occasionally encountered difficulties during diagnosis (*e.g.*, the possession of an incomplete family history), consultant haematologists will be queried during routine annual follow-up whether they have changed their initial diagnosis based upon information gleaned from subsequent clinical investigation. Only participants with unchanged diagnoses will be included for analyses.

6.2 Design: Outcomes, Exposures, Covariates & Analysis

In total, two series of three reports will be submitted to the WWEpi oncology division at GSK as part of the study. The first set of reports will centre on the retrospective investigation of the RLH UKITP Sub-Cohort while the second will encompass the entirety of retrospective and prospective data collected on combined study population. An analysis plan, which will be used as a foundation for these reports, is provided in Appendix A4-A1.

Exposure, outcome, and covariate status will be extracted from patient hospital medical records at haematology clinics where registration transpired. The validity of information contained in patient hospital medical records has been well documented in the epidemiological literature. Concern will rather rest with the extraction process itself, which will be centre-specific. While hospital medical records at the RLH will be interrogated on two separate occasions prior to extraction, it is likely that such a rigorous methodology will not be possible elsewhere. Extraction procedures at both the RLH and a random subset of collaborating centres will be subject to a validation study as detailed in Section 6.5 of this proposal.

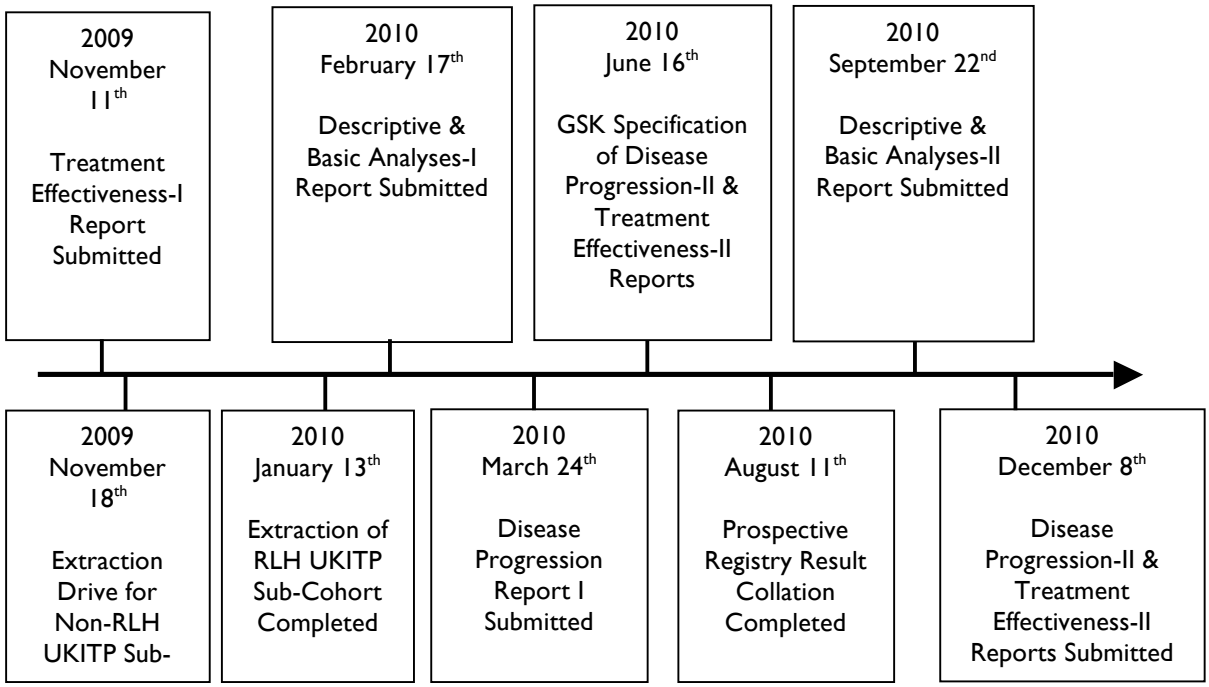
6.3 Adverse drug experience/event measures

N/A

6.4 Data collection and management

The timeline for the study is illustrated in Figure A4-2. All study-related information will be collected and managed by the lead epidemiologist and principal investigator. Non-anonymous participant-linked data will be stored on two secure, password-enables drives alone. All information accessed on external hardware or provided to GSK, third-parties, or the public will be fully-anonymised.

Figure A4-2: Data Extraction & Deliverables Timeline



6.5 Validation procedures

A validation study will be initiated mid-way through the second phase of the investigation. Random samples will be drawn separately from RLH and Non-RLH participants for whom data has been extracted. The hospital medical records of participants comprising these validation samples will be forwarded to a clinical consultant who will independently extract information to be contrasted with the original extractions performed by study personnel.

6.6 Sample size and power/precision calculations

The paucity of epidemiological information on primary ITP in adults introduces an additional element of uncertainty to the already inexact science of sample size estimation. Inspection of the participant numbers required to detect plausable ranges of effect sizes for the sampling of independent variables shown in Table A4-3 suggests that the cumulative study population will be insufficiently powered to detect co-morbid ITP associations with 80% power at a 0.05 level of signifance. The information gathered on disease burden will, however, still yield valuable descriptive information regarding both the incidence and prevalence of these conditions while potentially generating associative hypotheses for subsequent epidemiological testing. With regard to the areas of disease progression and treatment effectiveness, it is believed that these calculations are illustrative of the likely adeqauteness of the cumulative study population to uncover existing associations.

Table A4-3: Selection of Sample Size Calculations[13]

Variable	Adult Population Age	Population Incidence Rate/ Proportion	Incidence Rate/ Proportion Among Exposed	Detectable Shift (%)	Number of ITP Patients Required per Exposed/Unexposed Cohort $\alpha = 0.05$, $1-\beta = 0.80$
Cataracts	≥ 43	0.04[14]	0.05	25%	1892
			0.07	75%	257
Long-Bone Fractures-Men **Osteoarthritis Proxy** *Rate per person-year*	≥ 20	0.00076[15]	0.00095	25%	99,547
			0.00133	75%	13,519
Long-Bone Fractures-Female **Osteoarthritis Proxy** *Rate per person-year*	≥ 20	0.00046[15]	0.00059	25%	154,697
			0.00100	75%	11,171
Myocardial Infarction-Male *Rate per person-year*	< 65	0.00273[16]	0.00342	25%	27,522
			0.00479	75%	3,740
Myocardial Infarction-Female *Rate per person-year*	< 65	0.00066[16]	0.00083	25%	108,349
			0.00112	75%	17,629
Bleeding Events *Rate per person-year*	Not Specified	0.016[17]	0.008	50%	788
			0.004	75%	292
Mortality-Male *Proportion*	≥ 18	10.2%[18]	12.8%	25%	1,048
			17.9%	75%	125
Mortality-Female *Proportion*	≥ 18	6.3%[18]	7.9%	25%	1,950
			11%	75%	253
Platelet Counts- *Dichotomous* *(> 50 × 10^9/L)*	Not Specified	16%[19]	30%	88%	51
			50%	313%	11
Platelet Counts- *continuous* *(platelets/mm^3)*	Not Specified	25,000[19]	50,000 (sd: 15,000)	100%	8

6.7 Discussion

This study will effectively complement ongoing GPRD work on adult patients with primary ITP conducted by WWEpi. The validity of GPRD codes has been well documented in the epidemiological literature, and it is believed that the database will provide accurate, population-based estimates on the incidence, prevalence, and co-morbid burden of primary ITP in adults in the UK.[20]

Concern exists, however, as to the precise composition of the ITP population within the GPRD, particularly the prorportion of individuals warranting treatment consideration. While a diagnosis of primary ITP in adults may be indicated in the instance of platelet counts below 150 × 10^9/L, treatment guidelines put forward by the BCSH recommend intervention only in limited circumstances, namely cases of presentation with platelet counts below 30 × 10^9/L or symptomatic presentation with platelet counts below 50 × 10^9/L.[21] Documentation of platelet counts in adult patients with primary ITP in the GPRD is unfortunately sparse, with approximately one-third of this cohort having no reported platelet counts.[20]

Though admittedly stemming from a potentially innumerable genreral population denominator, segments of the study cohort will likely be well representative of the general and refractory populations of adults with primary ITP. Proposed analyses on this cohort will, therefore, yield valuable information on both natural and drug-induced co-morbidites, which may more accurately reflect the disease burden among the population to which eltrombopag/promacta may be marketed. Access to longitudinal platelet counts for all study participants moreover, presents the unique opportunity to uncover the progression of general and refractory, primary ITP among adults and the effectiveness of current ITP treatments within these populations. A summary of the potential strengths and limitations of utilising the study population are enumerated in Table A4-4.

Table A4-4: Potential Strengths and Limitations of the Study Population

Strengths	Limitations
will contain variable follow-up time (~1-30 years) on participants	will contain variable follow-up time (~1-30 years) on participants
will serve as one of the largest cohorts of adult patients with primary ITP ever assembled	the external validity of the study population will, as a whole, be potentially innumerable
primarily referred RLH UKITP Sub-Cohort will likely be well representative of the general population of adults with primary ITP	the sample size of the RLH UKITP Sub-Cohort is moderately small
secondarily referred RLH UKITP Sub-Cohort potentially will likely be well representative of the refractory population of adults with primary ITP	requires a significant and costly extraction effort
will contain longitudinal platelet counts on all patients, enabling unique analysis of • disease progression • treatment effectiveness • natural & treatment-induced	

7.1 Ethical approval and subject consent

The London Research Ethics Committee (REC) granted ethical approval in September 2009 for the collection of information corresponding to the field categories shown in Tables A4-5-7. Approval extended to non-anonymous data compilation, discussed at greater length in Section 7.2, DNA and RNA extraction and storage, genome-wide gene expression and SNP testing. All study participants will have provided informed written consent prior to enrolment, as documented by Appendices A4-A2 & A3.

7.2 Subject confidentiality

Approval for the compilation of non-anonymous data for the purposes of prospective tracking of study participants has been granted by the London REC. All printed or electronic information provided to third-parties or the public are required to be fully anonymised. Furthermore, no identifiable participant data will be accessed on GSK-owned hardware. The confidentiality of study participants will be fully respected in this regard.

7.3 Reporting of adverse drug events

N/A

7.4 Study closure/uninterpretability of results

N/A

7.5 Study milestones

Relevant study milestones are illustrated in Figure A4-2. The study commenced on October 17th, 2007 and will conclude in the fourth quarter of 2010. Over this period, two series of three reports will be submitted to the WWEpi oncology division at GSK.

Table A4-5: Participant Data for Extraction-I

Demographic Information: [Re-/Registration Form Only]

- Registration Date
- Patient Name, Date of Birth, Gender & Mailing Address
- Centre Name, Phone Number & Mailing Address
- Consultant Haematologist Name

Miscellaneous:

- Date of Diagnosis [Re-/Registration Forms Only]
- Date of Last Clinic Visit
- Platelet Count(s) (Count & Date)
- Weight at Diagnosis [Re-/Registration Forms Only]
- Change of ITP Diagnosis (Yes/No) [Annual Form Only]

Treatment: (Yes/No; Date(s), Dosage(s) & Duration(s) where applicable)

- Prednisolone
- IVIg
- Splenectomy (Laparoscopy/Laparotomy)
- Anti-D
- Methylprednisolone
- Dexamethasone
- Danazol
- Dapsone
- Azathioprine
- Cyclophosphamide
- Vinca Alkaloids
- Mycophenolate
- Plasmapheresis
- Protein A Immunoadsortion
- Interferon
- Cyclosporine
- Rituximab
- Platelet Transfusion
- Red Blood Cell Transfusion
- *H. pylori* Treatment
- Vitamin C Supplements
- Romiplostim
- Eltrombopag

Table A4-6: Participant Data for Extraction-II

Co-Morbid Conditions: (Yes/No, Date)

- Cataracts
- Osteoarthritis
- Type II Diabetes
- Hypertension
- Peptic Ulcers
- *H. pylori* Infection
- Renal Failure or Impairment
- Chronic Liver Disease
- Myocardial Infarction
- Ischaemic Stroke
- Transient Ischaemic Attack
- Deep Vein Thrombosis
- Pulmonary Embolism
- Splenomegaly
- Thyroid Disease
- Depression/Anxiety
- Miscarriage
- Cushing's Syndrome
- *Candida* Infection
- Pneumonia
- Other Autoimmune Disease
- Haematological Malignancy
- Solid Tumour Malignancy

Bleeding Events: (yes/no, date)

- Cutaneous bleeds
- Bleeds from the oral cavity
- Epistaxis
- Uterine bleeds
- Haematuria
- Gastrointestinal bleeds
- Intracranial haemorrhage
- Muscle bleeds
- Joint bleeds
- Subconjunctival bleeds
- Retinal bleeds

Table A4-7: Participant Data for Extraction-III

Biochemical Fields (Levels at Diagnosis) [Re-/Registration Only]

- Alanine Transaminase (ALT)
- Aspartate Transaminase (AST)
- Alkaline Phosphatase (ALP)
- Bilirubin

Haematological Fields [Re-/Registration Form Except Haemoglobin & Neutrophils]

- Haemoglobin (Levels at Diagnosis & Last Follow-Up)
- Neutrophils (Levels at Diagnosis & Last Follow-Up)
- White Blood Cells
- Red Blood Cells
- Mean Platelet Volume (MPV)
- Blood Group (A, B, AB & O; Rh Positive/Negative)
- Direct Agglutination Test (Yes/No; Positive/Negative)
- Marrow Aspirate (Yes/No; Conclusions)
- Trephine Biopsy (Yes/No; Conclusions)

Immunological Fields (Levels at Diagnosis) [Re-/Registration Form Only]

- Immunoglobulin
    - IgG
    - IgM
    - IgA
- Anti-Nuclear Antibodies

Coagulatory Fields (Levels at Diagnosis) [Re-/Registration Form Only]

- Prothrombin Ratio (PT)
- Activated Partial Prothrombin Time (APPT)
- Lupus Anticoagulant (LA)
- IgG-Anticardiolipin Antibody (aCL)
- IgG-Anticardiolipin Antibody (aCL)
- Reticulocyte Percentage

Indium Scanning ($t_{80\%}/t_{30\ minutes}$ spleen/liver ratio; date)

7.6 Study Advisory Committee

The study advisory committee will consist of the following individuals serving in the capacities specified. Progress updates will be submitted to the study advisory committee quarterly.

a) Dr. Dimitri Bennett (GSK)- administration, finance, planning & epidemiology

b) Dr. Mike Colopy (GSK)- biostatistics

c) Dr. Drew Provan (RLH)- administration, finance, planning & medicine

d) Professor Adrian Newland (RLH)- planning & medicine

f) Mr. Ameet Sarpatwari (Cambridge)- administration, finance, planning & epidemiology*

7.7 Study reporting and publications

The information contained in the study reports will be of interest to clinical haematologists, ITP patients, and the general public health community, presenting the potential for multiple conference presentations and peer-review manuscripts for publication. The principal investigator will be free to publish, present, and use study data as deemed fit, provided the provision of at least thirty-days for GSK to review and comment prior to submissions for third-party publication.

As reports stemming from this study will not specifically detail safety information regarding GSK marketed products, their inclusion in the Clinical Trial Registry is not mandated but will rather be at the discretion of the WWEpi oncology division.

7.8 Resourcing needs

The resources required to tackle this project are fully documented in Table A4-8. In brief, the undertaking will necessitate the full-time employment of one study coordinator and one data manager. The part-time services of a a database consultant, a web programmer, a statistical analyst, and a clinical specialist will additionally be enlisted. The remaining resources required consistute communications services, information technology (IT) products, and overhead expenditures. The total estimated cost for this project is £312,875.

Table A4-8: Resourcing Needs

Adult ITP Initiative Budget Proposal	Cost	Source
PI		
Conferences	2,000	Not Applicable
Travel		
Year 1	1,000	Not Applicable
Year 2 (5% Inflation Adjustment)	1,050	Not Applicable
Year 3 ((5% Inflation Adjustment)	1,103	Not Applicable
Study Coordinator		
PhD & Maintenance Expenses		
Year 1		
University Composition Fee	11,571	University of Cambridge
College Fee	2,001	University of Cambridge
Maintenance	14,300	Queen Mary University of London
Year 2 (5% Inflation Adjustment)		
University Composition Fee	12,149	University of Cambridge
College Fee	2,100	University of Cambridge
Maintenance	15,015	Queen Mary University of London
Year 3 (5% Inflation Adjustment)		
University Composition Fee	12,758	University of Cambridge
College Fee	2,205	University of Cambridge
Maintenance	15,765	Queen Mary University of London
Travel		
Year 1: Inter-UK Transport	500	Not Applicable
Year 2: Inter-UK Transport (5% Inflation Adjustment)	525	Not Applicable
Year 3: Inter-UK Transport (5% Inflation Adjustment)	551	Not Applicable
Year 2: International Conference Transport & Accommodation	500	Not Applicable
Year 3: As Above (5% Inflation Adjustment)	525	Not Applicable
Data Manager		
Salary		
Year 1	31,739	Queen Mary University of London
Year 2 (5% Inflation Adjustment)	33,326	Queen Mary University of London
Year 3 (5% Inflation Adjustment)	34,992	Queen Mary University of London
Travel		
Year 2: Inter-UK Transport	250	Not Applicable
Year 3: Inter-UK Transport (5% Inflation Adjustment)	263	Not Applicable
Year 2: International Conference Transport & Accommodation	500	Not Applicable
Year 3: As Above (5% Inflation Adjustment)	525	Not Applicable
Skills Development		
Year 2	500	Not Applicable
Year 3	500	Not Applicable
Mailing		
Stationary & Envelopes	2,500	Not Applicable
Postage	2,500	Not Applicable
Data Extraction		
Student Workers (£10 pounds/hours*10/hours/week*40 weeks*4	16,000	Not Applicable

students)		
Extraction Training	1,000	Not Applicable
Validation Study		
Independent Expert Clinical Analysis (£75/hour*40 hours*2 weeks)	6,000	Not Applicable
IT Services		
Database Services		
Remote Server & Front Page Design	15,000	Dendrite Ltd.
Annual Maintenance		
Year 2	1,500	Dendrite Ltd.
Year 3	1,500	Dendrite Ltd.
Miscellaneous Database Services		
*Year 1 (50 pounds per hour * 40 hours * 1 weeks)*	2,000	Dendrite Ltd.
Year 2 (5% Inflation Adjustment)	2,100	Dendrite Ltd.
Web Design Services		
*Web Construction (30 pounds per hour * 40 hours * 2 weeks)*	2,400	Association of Technology Staffing Companies: Freelance Web Designer
Troubleshooting		
Year 2 (30 pounds per hour * 40 hours * 2 weeks)	2,400	Association of Technology Staffing Companies: Freelance Web Designer
Year 3 (5% Inflation Adjustment)	2,520	Association of Technology Staffing Companies: Freelance Web Designer
Analytical/Statistical Services (£30/hour * 40 hours * 2 Weeks)	2,400	Yoh Index of Technology Wages 2006: SAS Programmer
Hardware		
Desktop-1	750	Dell
Desktop-2	750	Dell
Laptop	750	Dell
Multi-Function Colour Laserjet: Printer, Scanner, Phone, Fax	500	HP
Black & White Laser Printer (2x)	750	HP
Toners & Ink Cartridges	2,500	Not Applicable
Software		
SAS	400	Single User License-Queen Mary University of London
Various Software Site Licenses	1,000	Queen Mary University of London
Miscellaneous Office Equipment		
Year 1	2,000	Not Applicable
Year 2	2,000	Not Applicable
Year 3	2,000	Not Applicable
Publicity & Advertisement	5,000	Not Applicable
Overhead	36,038	Queen Mary University of London
Miscellaneous	404	Not Applicable
	312,875	

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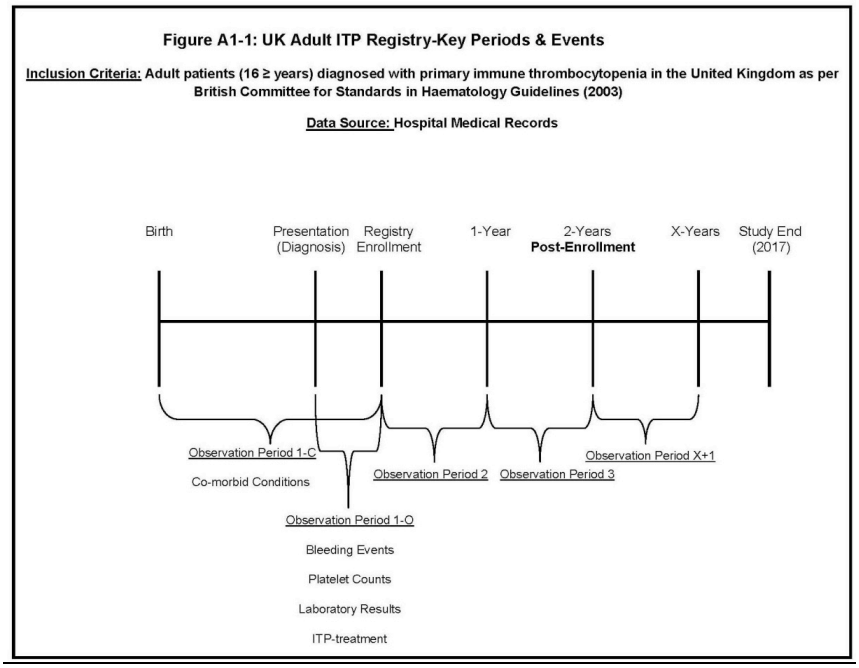
8. APPENDICES

Appendix A4-A1: Analysis Plan (Page 1 of 8)



Descriptive & Basic Analyses Report (Items I-IV)†‡

I. Baseline Demographics

a. summary: demographics at presentation

b. output: referral status (proportion & 95% confidence interval [CI]); gender (proportion & 95% CI); age-continuous at presentation (median & range); age-group at presentation (proportion & 95% CI); platelet count-continuous at presentation (median & range); platelet count-categories at presentation (proportion & 95% CI); initial ITP treatment within one month of presentation (proportion & 95% CI); wet bleeding at presentation (proportion & 95% CI); period prevalence of 24 co-morbid conditions at presentation (proportion & 95% CI)

c. stratifying variables: (1) none; (2) referral status; (3) referral status + gender (for all outputs except referral status & gender); (4) referral status + platelet count-categories at presentation (for all outputs except referral status & platelet count-categories at presentation) (5) referral status + age-dichotomous at presentation (for all outputs except referral status, age-continuous & age-categories at presentation); (6) referral status + gender + age-dichotomous at presentation (for all outputs except referral status, gender, age-continuous & age-group at presentation)

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II. **Platelet Progression**

a. summary: platelet count v. time graphs and documentation of platelet response (relative change of mean platelet count[§]) and disease relapse at 12 & 24 months post-diagnosis

b. output: platelet count v. time graphs, response (proportion & 95% CI) & disease relapse (proportion & 95% CI)

c. stratifying variables: (1) none; (2) referral status; (3) referral status + gender; (4) referral status + age-dichotomous at presentation; (5) referral status + ethnicity (6) referral status + common ITP treatment; (7) referral status + gender + age-dichotomous at presentation (for platelet count v. time graphs only); (8) referral status + gender + common ITP treatment (for platelet count v. time graphs only); (9) referral status + age-dichotomous at presentation + common ITP treatment (for platelet count v. time graphs only); (10) referral status + gender + age-dichotomous at presentation + common ITP treatment (for platelet count v. time graphs only)

III. Treatment Patterns

a. summary: ITP treatment frequency (initial ITP treatment; cumulative ITP treatment-modality; and cumulative ITP treatment-number)

b. output: initial ITP treatment-dichotomous within one month of presentation (proportion & 95% CI); common ITP treatment-categories (proportion & 95% CI); cumulative number of ITP treatments utilised by individual study participants (median & range)

c. stratifying variables: (1) none; (2) referral status; (3) referral status + gender; (4) referral status + age

IV. Co-Morbid Incidence[**]

a. summary: post-presentation & common ITP treatment incidence of co-morbid conditions

b. output: Incidence & 95% CI. Incidence will be provided using different time intervals (*i.e.*, 1-90 days; 1-180 days; 91-180 days; 181-360 days; 1-360 days; 1-2 years; Day 1- study end/follow up end). Assuming Index Date (D) stands for time of diagnosis and Index Date (T) stands for treatment start date, there may be Index Date(s) (T[A-Z]), where A-Z stand for each respective type of treatment (*i.e.*, IVIg, corticosteroids, splenectomy, and anti-D). Incidence will be provided as cumulative incidence (denominator is people: x out of y people, proportion & 95% CI) and incidence rates (denominator is person time, expressed per 10,000 person years & 95% CI). As this study is descriptive and exploratory in nature, there will be no formal hypothesis testing, rendering power calculations unnecessary. Precision of point estimates for co-morbid events will be calculated via 95% CIs. Statistical modeling may further be conducted for the purpose of hypothesis generation.

c. stratifying variables: (1) none; (2) referral status; (3) referral status + gender; (4) referral status + disease severity at presentation; (5) referral status + age-dichotomous at presentation; (6) referral status + common ITP treatment-dichotomous (for post-presentation only)

Appendix A4-A1: Analysis Plan (Page 3 of 8)

Modelling

Disease Progression Report (Items V & VI)

V. Multivariable Model for Determining the Likelihood of Major Bleeding Events†††‡‡

a. summary: development of prognostic model to a build risk score for major bleeding events
b. epidemiological category: disease progression
c. model(s): logistic regression
d. response variable: major bleeding events
e. predictor variables§§: age-continuous at presentation, gender, count-categories at presentation, wet bleeding at presentation, antinuclear antibodies at presentation, time since onset of disease, number of prior ITP treatments, splenectomy-status (other possible predictor variables maybe explored)

VI. Multivariable Model for the Risk of Thromboembolic Events***†††

a. summary: test of the relationship of thromboembolic events with (i) antiphospholipid antibodies at presentation (ii) co-morbid conditions, (iii) common ITP treatment and (iv) platelet count-categories at presentation for the time interval 0-24 months post-diagnosis
b. epidemiological category: co-morbid burden
c. model(s): Cox proportional hazards (or) Poisson Regression
d. response variable: thromboembolic events
e. predictor variables: gender, antiphospholipid antibodies at presentation, corticosteroid treatment, IVIg treatment, anti-D treatment, splenectomy status, hypertension, diabetes, chronic renal failure, prior TE, count-categories at presentation, age-continuous at presentation, mean platelet count- one month prior to thromboembolic event, haematological malignancy, solid tumour malignancy, smoking, inflammatory bowel disease (other possible predictor variables may be explored)

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Treatment Effectiveness Report (Item VII)

VII. Multivariable Model for Determining the Likelihood of Success from Splenectomy‡‡‡

a. summary: tests of the relationship between (a) autologous ^{111}In-labelled platelet sequestration pattern and (b) complete response at (i) 1-3 months (ii) 6-12 months and (iii) last follow-up post-splenectomy
b. epidemiological category: treatment effectiveness; prognostic model
c. model(s): Multivariable logistic regression (exploration of absolute & relative risk)
d. response variable: complete response (i) 1-3 months (ii) 6-12 months and (iii) last follow-up post-splenectomy
e. predictor variables: autologous ^{111}In-labelled platelet sequestration pattern, gender, age-continuous at splenectomy (other possible predictor variables maybe explored)

Note: Consultants of patients not undergoing splenectomy will be queried as to the reason(s) the procedure was not performed. Responses will be grouped into one of the following categories: test result, asymptomatic or mildly asymptomatic disease, patient choice, remission, co-morbid contraindication, other, and unspecified. Treatment usage among splenectomised and non-splenectomised patients at last follow-up will be compared. Fisher's Exact Test will be used to evaluate differences in the proportion of patients undergoing splenectomy within autologous ^{111}In-labelled platelet sequestration pattern strata and of males v. females lost and not-lost to follow-up as well as spenectomized and non-splenectomized patients. Student's T Test will be used to inspect age differences between patients lost and not-lost to follow-up as well as spenectomized and non-splenectomized patients.

Prospective Investigation

Note: In addition to a "Descriptive and Basic Analyses Report-II," the UK Adult ITP Team will work with GSK to develop two further study hypotheses in the second quarter of 2010, which will be addressed in deliverables "Disease Progression Report-II" and "Treatment Effectiveness Report-II." All three of these phase II reports will incorporate both retrospective and prospective data (annual prospective follow-up of patients enrolled in the Registry).

Appendix A4-A1: Analysis Plan (Page 5 of 8)

Table A1-1: Definition of Variables

Age-continuous	0-99 years
Age-dichotomous	0. < 50 years 1. ≥ 50 years
Age-group	A. 16-39 years B. 40-49 years C. 50-59 years D. 60-69 years E. 70-79 years F. ≥ 80 years
Anti-D treatment	0. no 1. yes
Antiphospholipid antibodies	*explanation: anticardiolipin (IgG or IgM) or lupus anticoagulant antibodies* 0. no 1. yes
Autologous ^{111}In-labelled platelet sequestration pattern	*explanation: spleen:liver ratio at time of 80% destruction over spleen:liver ratio at time of 30 minutes post-labelling; $x \leq 0.8$ hepatic, $0.8 < x \leq 1.4$ mixed; $1.4 < x \leq 2.0$ predominant splenic; $x > 2.0$ purely splenic* 0. purely splenic & predominant splenic 1. mixed & hepatic
Bleeding events	A. cutaneous B. oral cavity C. epistaxis D. uterine E. haematuria F. gastrointestinal G. intracranial haemorrhage H. muscle I. subconjunctival J. retinal

Appendix A4-A1: Analysis Plan (Page 6 of 8)

Co-morbid conditions[§§§]	cataracts peptic ulcer *H. pylori* infection renal failure or impairment chronic liver disease diabetes hypertension myocardial infarction ischaemic stroke transient ischaemic attack unstable angina deep vein thrombosis pulmonary embolism portal vein thrombosis hyperthyroidism hypothyroidism systemic lupus erythematosus Cushing's syndrome *Candida* infection haematological malignancy solid tumour malignancy phototoxicity splenomegaly anaemia death
Common ITP treatment-dichotomous	no yes
Common ITP treatment-categories	none corticosteroids intravenous immunoglobulin (IVIg) anti-D splenectomy
Complete response	*explanation: count above 100 × 10^9/L* *(NB: use of random-effects, repeated measures modelling may be explored)* no yes
Corticosteroid treatment	no yes
Chronic renal failure	no yes
Cumulative ITP treatment	0-10 treatments
Diabetes	no yes
Disease relapse	count < 50 × 10^9/L for ≥ 2 weeks (with or without bleeding/rescue) or < 50 × 10^9/L < 2 weeks with bleeding or rescue. no yes

Appendix A4-A1: Analysis Plan (Page 7 of 8)

Ethnicity	A. black B. east Asian C. mixed D. south Asian E. white F. other
Follow-up time (continuous)	0-360 person-months
Gender	0. no 1. yes
Haematological malignancy-dichotomous	0. no 1. yes
Haematological malignancy-categories	A. acute myeloid leukaemia B. chronic lymphocytic leukaemia C. chronic myeloid leukaemia D. hairy cell leukaemia E. non-Hodgkin's lymphoma F. Hodgkin's lymphoma G. myeloma
Hypertension	0. no 1. yes
Inflammatory bowel disease	0. no 1. yes
Initial ITP treatment-dichotomous	0. no 1. yes
Initial ITP treatment-categories	A. none B. corticosteroids C. IVIg D. anti-D
ITP treatment	A. none B. prednisolone C. IVIg D. splenectomy E. anti-D F. methylprednisolone G. dexamethasone H. danazol I. dapsone J. azathioprine K. rituximab L. cyclophosphamide M. vinca alkaloids N. mycopehnolate O. cyclosporine P. platelet transfusion Q. plasmapheresis R. protein A immunoadsortion S. interferon T. *H.* pylori eradication (triple-therapy) U. eltrombopag V. romiplostim
IVIg treatment	0. no 1. yes
Major bleeding events	*explanation: Common Terminology Criteria for Adverse Events (CTCAE)-v4 grade 3 or higher bleed* 0. no 1. yes

Appendix A4-A1: Analysis Plan (Page 8 of 8)

Platelet count-continuous	1-1,500 × 10^9/L
Platelet count-categories	0. < 10 × 10^9/L 1. 10 × 10^9/L ≤ X < 20 × 10^9/L 2. 20 × 10^9/L ≤ X < 30 × 10^9 /L 3. 30 × 10^9/L ≤ X < 50 × 10^9/L 4. 50 × 10^9/L ≤ X < 150 × 10^9/L 5. 150 × 10^9/L ≤ X < 400 × 10^9/L 6. ≥ 400 × 10^9/L
Platelet count-response	*explanation: count* ≥ 30 and at least doubling of the baseline count *(NB: use of random-effects, repeated measures modelling may be explored)* 0. no 1. yes
Referral status	0. general practitioner referred 1. haematologist referred
Solid Tumour Malignancy-dichotomous	0. no 1. yes
Solid Tumour Malignancy-categories	A. lip, oral cavity and pharynx B. digestive organs C. respiratory & intrathoracic organs D. bone & articular cartilage E. skin F. connective and soft tissue G. breast & female genital organs H. male genital organs I. urinary organs J. nervous system K. endocrine glands & related structures
Smoking	0. no 1. yes
Splenectomy status	0. no 1. yes
Thromboembolic events (TE)	A. none B. arterial TE i. myocardial infarction ii. ischemic stroke iii. transient ischemic attack iv. unstable angina v. other arterial TE C. venous TE i. pulmonary embolism ii. deep vein thrombosis iii. portal vein thrombosis D. other venous TE
Wet bleeding	0. no 1. yes

Appendix A4-A2: Study Informed Consent Agreement

UK Adult ITP Registry

Please initial boxes.

1. I confirm that I have read and understand the Prospective Participant Overview of the UK Adult ITP Registry dated 20.08.09 (version 2.6) and have had the opportunity to address any questions or concerns that I had concerning the study. ☐

2. I understand that my participation is voluntary and that I am free to withdraw from the study at any time without giving any reason and without my medical care or legal rights being affected. ☐

3. I understand that ITP-related information from my medical notes may be extracted by responsible individuals comprising my clinical care team or the chief investigator's study team. I give permission for these individuals to have access to my hospital records. ☐

4. I allow the study coordinator for the UK Adult ITP Registry to share fully-anonymised data with GlaxoSmithKline Research Ltd. and the Paediatric and Adult Intercontinental Registry on Chronic ITP (PARC-ITP) Study annually during the investigation. I understand that, as part of these collaborations, data will be shared with researchers in non-European Economic Area (EEA) countries which may not have laws protecting patient privacy to the same extent as the UK Data Protection Act or European Law. Within these constraints, I am aware that study personnel will take all reasonable steps to protect my privacy. ☐

5. I agree to take part in the U.K. Adult ITP Registry ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person Taking Consent (if different from researcher)	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

Appendix A4-A3: Subsequent Tissue Usage Informed Consent Agreement

UK Adult ITP Registry

Please initial boxes.

1. I consent to the storage of my blood or saliva in a research tissue bank for use in future studies. ☐

2. I agree that the blood or saliva I have supplied may be used for future genetic research but not for research involving cloning or for the testing of inherited diseases without my express consent. ☐

3. I am aware that I am free to withdraw my consent for the subsequent storage and use of my blood or saliva at any time. ☐

If you have any preferences or exclusions for use of your donated blood, or any other comments, please include them here:

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person Taking Consent (if different from researcher)	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature