Infectious and bleeding complications in patients with hematological malignancies

Studies on diagnosis and prevention

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Abstract
Svensson, T. 2017. Infectious and bleeding complications in patients with hematological malignancies. Studies on diagnosis and prevention. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1304. 49 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9830-6.

The overall aim of this thesis is to improve knowledge about the prevention of infectious and bleeding complications in patients with hematological malignances, primarily in those with chronic lymphocytic leukemia (CLL) and myelodysplatic syndrome (MDS).

Hypogammaglobulinemia, impaired production of immunoglobulins (Ig), is an established risk factor for infection, but the impact of IgG pure subclass deficiency (IgG subclass deficiency with adequate production of IgG, IgA, and IgM) has been debated. In a retrospective single institution study, we concluded that pure IgG subclass deficiency in CLL patients is rare and is not associated with an increased risk of infection. Hence, routine analysis of IgG subclasses in patients with CLL is not warranted.

There is no consensus on recommending vaccination against *Streptococcus pneumoniae* to CLL patients mainly because comparative studies are lacking. In our randomized trial, the efficacy of a conjugated pneumococcal vaccine on immune response was superior or equal to a polysaccharide vaccine for all pneumococcal serotypes common for the two vaccines. A conjugate pneumococcal vaccine should therefore be included in vaccination programs for patients with CLL.

Bronchoalveolar lavage (BAL) is a well-established invasive method to identify the cause of pulmonary infiltrates in immunocompromised patients. In a retrospective trial, we have studied the diagnostic yield of BAL in patients with hematological malignancies. We concluded that BAL is highly useful in either verifying or excluding some of the important respiratory tract infections affecting these patients, particularly invasive pulmonary aspergillosis (IPA) and Pneumocystis jirovecii pneumonia (PJP). However, standardized procedures for BAL sampling should be continually revised to avoid unnecessary microbiological tests.

Thrombocytopenia, an adverse prognostic factor in patients with MDS, can be aggravated by azacitidine, first-line treatment for high-risk MDS. Eltrombopag, a thrombopoietin-receptor agonist (TPO-R), alleviates thrombocytopenia in patients with immune thrombocytopenic purpura (ITP). In a phase I clinical trial, we concluded that the combination of eltrombopag and azacitidine in high-risk MDS patients with thrombocytopenia is feasible and well tolerated in doses up to 200 mg eltrombopag daily.

Keywords: Chronic lymphocytic leukemia, Immunodeficiency, Hypogammaglobulinemia, IgG subclass, Pneumococci, Pneumococcal vaccine, Polysaccharide vaccine, Protein-conjugate vaccine, Aspergillosis, Bronchoalveolar lavage, Invasive fungal disease, Pneumocystis jirovecii pneumonia, Myelodysplastic syndrome, Azacitidine, Eltrombopag, Thrombocytopenia, Thrombopoietin receptor

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I   **Svensson, T**., Höglund M., Cherif H. (2013) Clinical significance of serum immunoglobulin G subclass deficiency in patients with chronic lymphocytic leukemia. *Scandinavian Journal of Infectious Diseases*, 45:7, 537-542

II   **Svensson, T**., Kättström, M., Hammarlund, Y., Roth, D., Andersson, P-O., Svensson, M., Nilsson, I., Rombo, L., Cherif, H., Kimby, E. Conjugated pneumococcal vaccine triggers a better immune response than polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia – a randomized study by the Swedish CLL group. *Manuscript*

III   **Svensson, T**., Lamberg-Lundström, K., Höglund, M., Cherif, H. (2016) Utility of bronchoalveolar lavage in diagnosing respiratory tract infections in patients with hematological malignancies: are invasive diagnostics still needed? *Upsala Journal of Medical Sciences*, Oct 14:1-5

IV   **Svensson, T**., Chowdhury, O., Garelius, H., Lorenz, F., Saft, L., Jacobsen, S-E., Hellström-Lindberg, E., Cherif, H. (2014) A pilot phase I dose-finding safety study of the thrombopoietin receptor agonist, eltrombopag, in patients with myelodysplastic syndrome treated with azacitidine. *European Journal of Haematology*, 93(5):439-45

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Contents

Abbreviations

AE	Adverse event
Allo SCT	Allogeneic stem cell transplantation
AML	Acute myeloid leukemia
ASH	American Society of Hematology
Aza	Azacitidine
ANC	Absolute neutrophil count
BAL	Broncho alveolar lavage
BG	1,3-beta-D-glucan
BM	Bone marrow
CLL	Chronic lymphocytic leukemia
CMML	Chronic myelomonocytic leukemia
CMV	Cytomegalovirus
COP	Cryptogenic organizing pneumonia
CR	Complete response
CT	Computer tomography
DAT	Direct antiglobulin test
DLT	Dose limiting toxicity
DMC	Data monitoring committee
DVT	Deep vein thrombosis
EBV	Epstein Barr virus
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked immunosorbent assay
ESA	Erythropoietin stimulating agents
IFD	Invasive fungal disease
G-CSF	Granulocyte colony stimulating factor
GM	Aspergillus galactomannan
GMC	Geometric mean concentration
GMR	Geometric mean ratios
GMT	Geometric mean titer
GVHD	Graft versus host disease
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem and progenitor cell
Ig	Immunoglobulin
IgG4-RD	IgG4 related disease
Int-1	Intermediate 1
Int-2	Intermediate 2

IPA	Invasive pulmonary aspergillosis
IPSS	International prognostic scoring system
IPPS-R	International prognostic scoring system-revised
ITP	Immune thrombocytopenic purpura
IVIg	Intravenous immunoglobulin
IWG	International Working Group
LLOQ	Lower limit of quantification
MDS	Myelodysplastic syndrome
MTD	Maximum tolerable dose
NGS	Next generation sequencing
OPA	Opsonophagocytic assay
PB	Peripheral blood
PCR	Polymerase chain reaction
PCV	Protein-conjugate pneumococcal vaccine
PCV13	13-valent protein-conjugate pneumococcal vaccine
PJP	Pneumocystis jirovecii pneumonia
PPSV23	23-valent polysaccharide pneumococcal vaccine
PSB	Protected specimen brush
RBC	Red blood cell
SAE	Serious adverse event
sAML	Secondary acute myeloid leukemia
SCT	Stem cell transplantation
SOP	Standard operating procedure
TPO	Thrombopoietin
TPO-R	Thrombopoietin receptor
WHO	World Health Organization

Introduction

Hematological malignancies

Hematological malignancies are a group of heterogeneous diseases with varying clinical pictures and prognoses that have in common a clonal expansion of immature malignant hematopoietic cells. Infiltrated by the expanding malignant clone, the bone marrow (BM) fails to produce sufficient blood cells, leading to anemia, leukopenia, and thrombocytopenia, key features of most hematological malignancies. Furthermore, chemotherapy and other treatments may lead to a temporarily worsening of BM failure as well as suppression of the immune system. Anemia, low red blood cell (RBC) count, causes fatigue and can be treated with RBC transfusions or erythropoietin-stimulating agents (ESA). Neutropenia can, in many cases, be shortened using granulocyte colony stimulating factors (G-CSF), and patients with severe thrombocytopenia can benefit from platelet transfusions (1-6).

Infectious complications in hematological malignancies

A clinical challenge for hematologists is the increased risk of infection seen to varying degrees in patients with hematological malignancies. The etiology behind this is multifactorial; neutropenia, particularly if severe and sustained, predisposing to serious and even lethal infections, is commonly temporarily aggravated after treatment with cytostatic agents. Patients with severe neutropenia, an absolute neutrophil count (ANC) below 0.5 x 10^9/L, frequently suffer from bacterial infections causing febrile neutropenia, the most common clinical presentation. Invasive procedures (e.g., the frequent use of central venous lines) add to this vulnerability. Chemotherapy as well as certain immunotoxic drugs, also leads to an increased susceptibility to infection through damaging of T-cells and mucous membranes (7-10). In particular, allogeneic stem cell transplantation (allo SCT) is associated with an extended period of immunodeficiency because of a slow immunological reconstitution. Sustained decrease in immune response predisposes patients to opportunistic infections such as *Cytomegalovirus* (CMV), *Epstein Barr virus* (EBV) and Aspergillosis, and other fungal infections (9, 11-13). Hypogammaglobulinemia, serum levels of immunoglobulins below normal

values, is a well-known immune defect in this patient group, especially in patients with B-cell malignancies (14-17).

Chronic Lymphocytic Leukemia

CLL is characterized by clonal proliferation and the accumulation of neoplastic B-cells with prolonged survival and resistance to apoptosis. The diagnosis of CLL requires at least 5×10^9 monoclonal lymphocytes/L in peripheral blood (PB) in consecutive tests over at least three months and a typical immunophenotype by flow cytometry. The incidence is 4–7/100 000, making it the most common form of leukemia in the western world, comprising about 30% of all new cases (18-22). The disease, which primarily affects elderly people, is heterogeneous, and prognosis and treatment differ widely between patients. New cases are often diagnosed when the disease is still indolent and those patients are monitored rather than treated. The criteria for initiating treatment are anemia and/or thrombocytopenia caused by BM failure, progressive lymphocytosis and massive or progressive spleen and/or lymph nodes, and B symptoms such as weight loss, fatigue, fever, and/or night sweats (20, 23-26). CLL is associated with an increased risk of infection, which is a major cause of morbidity and mortality in these patients. Reasons for this include T- and NK-cell dysfunction, complement defects, defective granulocyte function and neutro-/monocytopenia, and hypogammaglobulinemia. Short- and long-term side effects of treatment with chemo-immunotherapy and new, targeted agents may further increase susceptibility to infection (18, 27-29).

Hypogammaglobulinemia and IgG subclass deficiency in CLL

Hypogammaglobulinemia (serum levels of immunoglobulins (Ig) A, G, and/or M below normal values) is common in CLL. The reason for this is an inability to produce adequate immunoglobulins due to lack of functional B-cells. Any combination of Ig deficiency may occur. The reported incidence of hypogammaglobulinemia in newly diagnosed and untreated CLL patients varies from 10% to 45%, but is increasing over time, leading to a reported prevalence of up to 50% to 70% in unselected patient materials. Although the association between serum levels of particularly IgG below normal range and a predisposition to infectious complications is established, hypogammaglobulinemia has never been shown to be an independent risk factor for an unfavorable long-term outcome (15, 30-35).

Subclasses IgG1, IgG2, IgG3, and IgG4 account for 60–65%, 20–25%, 5–10%, and 3–6% of the total amount of IgG (36), and patients with CLL generally have lower IgG subclass levels than healthy controls (31, 37, 38) (Table 1). The differences in function of the four subclasses are not fully understood, but they show considerable differences in their bioactivities. IgG2, for

instance, dominates in the defense against capsulated bacteria such as *Streptococcus pneumoniae*. IgG1 and IgG3 predominantly respond to protein antigens such as viruses, and IgG4, the least abundant in serum, has recently become the topic of intense interest as it is linked to so called IgG4 related diseases (IgG4-RD) affecting organs such as pancreas and the salivary glands (39-42). It is not known whether pure IgG subclass deficiency (IgG, IgA, and IgM within normal range) leads to an increased risk of infectious complications in CLL patients.

In patients with CLL-associated hypogammaglobulinemia and repeated episodes of severe infection, the clinical value of substituting intravenous immunoglobulin (IVIg), which consists mainly of IgG pooled from donated plasma, is well documented, although its use in clinical practice varies between centers. It is important to remember that recommendations are based on studies that date from the 1980s and 1990s, long before modern CLL therapies were available (14-16, 33, 43-45). There is no consensus on whether individuals with pure IgG subclass deficiency should receive IVIg. Consequently, serum levels of immunoglobulins (i.e., IgG, IgA, and IgM) are routinely analyzed in clinical practice, whereas serum levels of the subclasses of IgG usually are not (18, 46).

Table 1. Incidence of IgG subclass deficiency in patients with CLL

Reference	Patient population	Outcomes
Copson et al. (38)	9 unselected CLL patients (2 previously treated) compared to 9 healthy controls	Total IgG, IgG3, and IgG4 were lower than in healthy controls. No difference was seen in IgG1 and IgG2 between the two groups.
Lacombe et al. (37)	52 unselected CLL patients (13 previously untreated)	Total IgG, IgG1, IgG2, and IgG3 under lower normal value in 39%, 39%, 19%, and 21% of the patients, respectively. All possible combinations of IgG subclass and Ig class deficiencies were observed.
Freeman et al. (31)	150 unselected CLL patients	Total IgG, IgG1, IgG2, IgG3, and IgG4 under lower normal value in 27%, 28%, 19%, 52%, and 23% of the patients, respectively.

Vaccination in CLL

Vaccination is a straightforward option in raising specific immunity and reducing infections. However, patients with CLL and other B-cell malignancies are known to respond poorly to traditional polysaccharide vaccines, with immunization response rates in unselected CLL patients varying from 0 to 22% in different studies (12, 15, 47-52). The reasons for this are multifactorial, but impaired humoral immunity (inadequate production of specific antibodies after vaccination due to a lack of functional B-cells) and defects in T-cells and the complement system are crucial. The response rate to vaccination has been shown to be better in an early stage of the disease, when the immune system is less affected, and before treatment has been initiated (12, 48, 49, 53-57).

Conjugation of polysaccharide protein carriers (so-called protein-conjugate vaccines [PCVs]) renders a thymus-dependent, memory-inducing, more immunogenic vaccine (53, 58-60). Although poorly investigated, conjugate vaccines may, according to a few studies, render a higher immune response than polysaccharide vaccines in patients with CLL (47, 48, 53, 56, 57).

Infections caused by *Streptococcus pneumoniae* are a major cause of morbidity and mortality in CLL patients, making its prevention essential (47, 55, 58, 61). More than 90 serotypes of *S. pneumoniae* have been identified, but the vast majority of human disease is caused by fewer than 30. Modern PCVs cover 13 pneumococcal serotypes (PCV13), whereas the polysaccharide vaccine (PPSV23) covers 23 different ones (61, 62). Protein-conjugate pneumococcal vaccines were first approved for infants and immunodeficient patients but are now also administered to the elderly. Their superior efficacy over polysaccharide vaccines is well established in children and they are globally recommended for routine childhood immunization. PCVs have dramatically decreased the incidence of invasive pneumococcal disease, but a shift in the distribution of disease causing serotypes has followed (59, 60, 62-68). In patients with CLL, randomized studies comparing the two different types of pneumococcal vaccines are lacking, and consequently there is no consensus on vaccination recommendations (53, 69).

Pulmonary infection in hematological malignancies

The range of pathogenic microorganisms causing pulmonary infections in patients treated for hematological malignancies, in particular those undergoing allo SCT, is broad and includes such opportunistic microorganisms as *Aspergillus* spp., *Candida* spp., *Mycobacterium* spp., *Pneumocystis jirovesii*, and respiratory viruses (70-74). In patients with severe neutropenia related to hematological disease or its treatment, 40% to 60% develop pulmonary infiltrates (71, 75, 76), and pneumonia is the primary cause of mortality not di-

rectly related to the hematological malignancy itself. However, in the majority of these cases the causative pathogen remains undetected (73, 74, 77, 78). Although computer tomography (CT) chest scan is a highly sensitive method, its results are often not specific due to a decreased inflammatory response and similarities in findings caused by different types of atypical pneumonias (71, 75, 76).

Broncho alveolar lavage

Broncho alveolar lavage (BAL) is a well-established diagnostic method for identifying the causative microorganism in patients with respiratory symptoms and pulmonary infiltrates. In the procedure, which is usually performed by a pulmonologist, a flexible bronchoscope is passed through the mouth into the bronchial tree. Fluid is squirted into a small part of the lung and then collected for examination (Figure 1). A protected specimen brush (PSB) can be used to obtain a more representative sample material. Infrequent but serious complications include bleeding and pneumothorax and respiratory failure, but procedure-related mortality is nevertheless extremely rare. Microbiological tests often based on samples collected from BAL include cultures for bacteria (including mycobacteria) and fungi, polymerase chain reaction (PCR) analyses for *Aspergillus* spp., CMV, *Legionella* spp., respiratory viruses, mycobacteria, and *Pneumocystis jirovecii*, and immunofluorescence for *Pneumocystis jirovecii* (71, 79, 80).

The diagnostic yield from BAL in patients with hematological malignancies varies from 15% to 90% in different studies and subpopulations (71, 75-77, 81-83); the earlier the investigation after symptom onset, the greater the likelihood of finding the causative pathogen (72, 81). Non-invasive methods for diagnosing respiratory infections such as invasive fungal disease (IFD) are under constant improvement and invasive methods such as BAL need to be continually re-evaluated (70, 84-88).
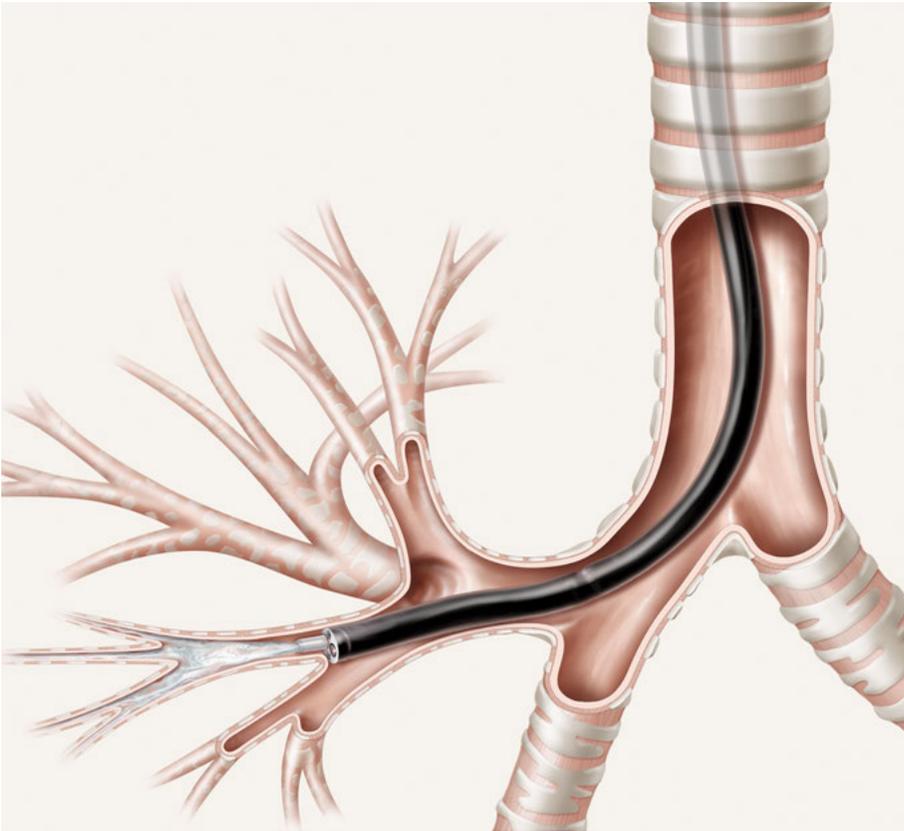
Figure 1. Illustration of the BAL procedure, courtesy of OLYMPUS

Bleeding and thrombocytopenia in hematological malignancies

Thrombocytopenia (i.e., low platelet levels) is a significant manifestation of many hematological malignancies and can be further aggravated by chemo-immunotherapy. Thrombocytopenia leads to increased risk of bleeding and can, in severe cases, be life-threatening. It is commonly treated and/or pre-vented by platelet transfusions (2, 5, 89).

Myelodysplastic syndromes

Myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal hematological malignancies characterized by ineffective hematopoiesis, progressive BM failure, and an increased risk of transformation to acute myeloid leukemia (AML). Prognosis is estimated on the International Prog-nostic Scoring System (IPSS). Patients with high-risk MDS (IPSS Int-2 and

high-risk) constitute one third of newly diagnosed MDS patients and have very poor prognoses, with a median overall survival of 0.4 to 1.2 years, whereas patients with low-risk MDS (IPSS Low and Int-1) have a better prognosis with a median overall survival of 3.5 to 5.7 years (90-93). A revised prognostic scoring system (IPSS-R) introduced in 2012 attributes a higher impact to cytogenetic aberrations (94). The recent introduction of next generation sequencing (NGS) in the diagnostic work-up of MDS has improved our understanding of the disease and is expected to further refine the prognostic scoring system (95).

Patients with high-risk MDS not eligible for the only curative treatment modality, induction chemotherapy and allo SCT (96, 97), are usually treated with the hypomethylating agents azacitidine (Aza) or decitabin, that have been shown to prolong overall survival, reduce the risk of leukemic transformation, and improve quality of life (98-101). Low-risk MDS can be treated with blood transfusions (and subsequent iron chelation therapy to reduce iron overload), ESA, and G-CSF (102-104).

Thrombocytopenia in MDS

Thrombocytopenia is a significant clinical manifestation of MDS and an independent adverse risk factor for survival. The mechanisms of thrombocytopenia in MDS have yet to be fully elucidated, but suppression of megakaryocytic differentiation by inhibitory cytokines, enhanced apoptosis, and defective responses to growth factors are postulated to be responsible. Increased platelet consumption, through immune and nonimmune mechanisms, has also been reported in MDS (89, 105, 106). Complications secondary to thrombocytopenia contribute to significant morbidity and mortality. Lethal hemorrhagic complications occur in 14% to 24% of cases (105-107). Furthermore, thrombocytopenia can deteriorate during treatment with chemotherapy or hypomethylating agents. In the pivotal randomized studies with Aza, severe (Grade 3–4) thrombocytopenia was reported in up to 85% of patients, and prolonged thrombocytopenia was the main cause of Aza dose reductions, treatment delays, or discontinuation (89, 98, 99). Currently, platelet transfusions are the only reliable option for severe and symptomatic thrombocytopenia in MDS. However, this provides only transient benefit and is limited by allo-immunization, high costs, limited availability, and (although uncommon) infectious complications (89, 105-107).

Thrombopoietic stimulating agents

Thrombopoietin-receptor (TPO-R, also known as c-MPL) agonists are available in clinical practice, approved mainly for the second- or third-line treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP). Two TPO-R agonists both lead to increased platelet production,

but with different modes of action: romiplostim is a subcutaneously administered peptibody that directly activates the c-MPL and eltrombopag is an orally administered non-peptide small-molecule that binds to the transmembrane region of c-MPL activating downstream signaling pathways (Figure 2) (89, 108-114). Their safety and efficacy in alleviating thrombocytopenia in patients with other hematological malignancies, including MDS, is not clear. Romiplostim has been shown to increase platelet counts and decrease the need for platelet transfusions in thrombocytopenic MDS patients. However, one study, evaluating the effect of romiplostim in low-risk MDS patients, showed a trend toward disease progression to AML and was stopped prematurely. Although this finding could not be confirmed on long-time follow-up, it has prevented further studies. In contrast, no connection between eltrombopag and premature transformation to AML has been shown. The few studies that have evaluated eltrombopag as monotherapy and as concomitant treatment with chemotherapy in patients with AML and MDS are summarized in Table 2 (89, 108, 115-118).
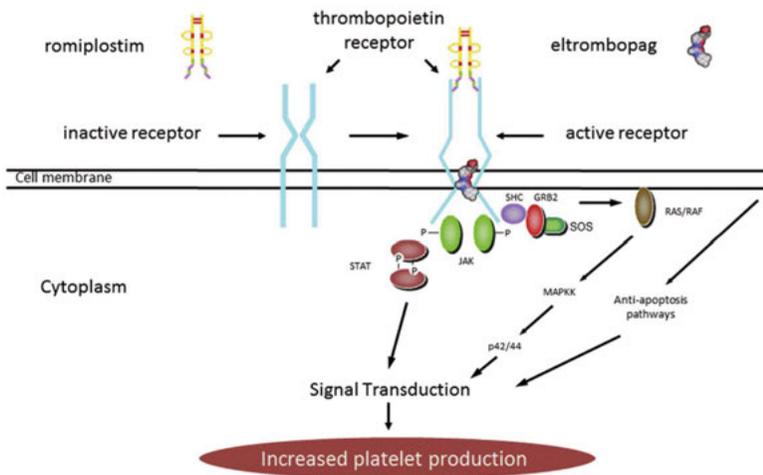

Figure 2. TPO-R agonist mechanism of action. Romiplostim and eltrombopag bind the TPO-R at different sites. Romiplostim binds the TPO-R at the same distal cytokine homology region as TPO and eltrombopag binds in the transmembrane region, courtesy of Best Practice & Research Clinical Haematology

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Table 2. Summary of eltrombopag clinical trials

Reference	Number of patients	Interventions	Outcomes
Mittelman et al. (119)	17 patients with IPSS intermediate-2 or high-risk MDS and AML	Single arm study of eltrombopag in dose escalation from 100 to 300 mg every two weeks	During eltrombopag treatment, a reduction in need for platelet transfusions was seen in 59% of the patients and a platelet response was observed in 24%. No change in BM-blast count after initiation of eltrombopag treatment was observed.
Oliva et al. (120)	90 patients with IPSS low or intermediate-1 risk MDS with thrombocytopenia $<30\times10^9$/L	Patients were randomized 2:1 to eltrombopag (dose escalation from 50 to 300 mg every two weeks) or placebo for at least 24 weeks and until disease progression	With a median follow-up time of 11 weeks, a platelet response was seen in 47% of the patients in the eltrombopag group versus 3% in the placebo group. There were more bleeding events in the placebo arm (42%) than in the eltrombopag arm (14%). No difference in the incidence of AEs or in AML progression was observed.
Platzbecker et al. (121)	98 patients with advanced MDS or AML ineligible for anti-leukemic therapy with thrombocytopenia $<30 \times 10^9$/l	Patients were randomized 2:1 to eltrombopag (dose escalation from 50 to 300 mg every two weeks until platelet response) for 6 months.	38% percent in the eltrombopag group achieved platelet transfusion independence for $\geqslant$ 8 weeks vs. 21% in the placebo group. Severe hemorrhages ($\geqslant$ Grade 3) were also less frequent in the eltrombopag group, although not statistically significant. No increase in BM blasts was seen in the eltrombopag arm.

The hematopoietic stem cell and MDS

It is well established that normal hematopoietic stem cells (HSCs) are the top of the hierarchy of the hematopoiesis and have the capacity for self-renewal and multilineage differentiation (122, 123). They are characteristically quiescent and reside in the BM, but can enter cell cycle when needed (123). There is substantial evidence that a similar hierarchy is applicable in cancer development, the cancer HSC being the obvious target for cancer treatment (124).

Thrombopoietin (TPO) has a critical role in the maintenance, expansion, and cycling of normal HSCs (125-127). The effects of TPO-R stimulation in patients with MDS and other myeloid malignancies are mostly unknown. TPO-R agonists may have positive effects such as tumor inhibition and, by inducing the cancer HSCs to enter cell cycle, making them more vulnerable to chemotherapy, but they may also stimulate malignant proliferation. It is therefore essential to study the impact of all TPO-R agonists on primitive BM populations. The direct effects of eltrombopag on the hematopoietic stem and progenitor cells (HSPCs) of patients with MDS is largely unknown; however, studies on cell lines have shown that it inhibits the proliferation of leukemic cells, probably through a TPO-R independent mechanism (128-132).

Aims

The overall aims of this thesis are to improve our knowledge of preventing infectious and bleeding complications in patients with hematological malignancies, primarily in patients with CLL and MDS. The specific aims of the studies:

- To assess the prevalence of IgG subclass deficiency and its correlation to increased risk of infectious complications in patients with CLL and to investigate the clinical value of routine measurement of serum IgG subclass levels.
- To determine whether immune response in patients with CLL improves more from vaccination with a 13-valent conjugated pneumococcal vaccine, Prevenar13 (PCV13), than with a conventional 23-valent capsular polysaccharide vaccine (PPSV23).
- To investigate the diagnostic yield and clinical value of BAL in patients with hematological malignancies and suspected pulmonary infection.
- To explore the safety and tolerability of combining eltrombopag with Aza as a means of alleviating thrombocytopenia in patients with MDS and to assess the potential effects of eltrombopag on HSPCs.

Methods

This section provides a summary of the experimental methods used in this thesis; for further details see the respective Papers I–IV.

Paper I

At a given point in 2011 all adult patients (n = 202) at the Uppsala University Hospital with a CLL diagnosis, regardless of the time since their diagnosis or previous CLL treatment (if any), were identified through local patient registers and asked to participate in the study. Serum levels of Ig and IgG subclasses from 146 of these patients were analyzed after informed consent was obtained. Patients' records and questionnaires were retrospectively reviewed for their frequency and types of infection.

Serum levels of Ig were analyzed with capillary electrophoresis and IgG subclasses (IgG1, IgG2 and IgG3) were analyzed with kinetic nephelometry (133). Hypogammaglobulinemia and IgG subclass deficiency were defined as values below normal range as per definition by the Uppsala University Hospital laboratory. Pure subclass deficiency was defined as subclass deficiency of IgG without hypogammaglobulinemia (total IgG, IgA, and IgM within normal range).

All infectious episodes since the diagnosis of CLL that required treatment of any kind (antibiotics, antimycotics or antiviral therapy) were recorded, whereas minor infections such as common colds were not. Infections were grouped as (1) all infections (any type of infection requiring treatment), (2) severe infections (requiring inpatient treatment), and (3) sepsis (severe infection with microbiologically verified bacteremia). Important disease-related factors, and factors possibly associated with an increased risk of infection were extracted from the records; these included age, gender, stage of disease at diagnosis, duration of CLL, previous treatment for CLL, and allo-SCT.

Statistical analyses of event rates were performed using an adjusted Poisson regression model. In the comparison of baseline characteristics between groups, Wilcoxon rank-sum test was used for continuous variables and Fisher exact test for categorical variables.

Paper II

In this randomized, non-blinded trial, 128 treatment naïve CLL patients from eight hematology clinics in Sweden were randomized with equal probability to receive vaccination with PCV13 (n = 63) or PPSV23 (n = 65) (Figure 3). Patients were stratified by IgG levels and CLL stage (using the Rai clinical staging system), because of expectation of a lower immunization response in patients with low serum IgG levels and clinically advanced disease. Major exclusion criteria were intention to start CLL treatment within one month, other malignancies, allergic reaction to vaccines in the past, neutropenia (ANC below 0.5 x10^9/L), positive direct antiglobulin test (DAT) or known previous hemolysis, previous vaccination with pneumococcal vaccine within 5 years, and ongoing infection. Adverse events (AEs) were monitored throughout the first month and serious adverse events (SAEs) throughout the first six months after vaccination.

Blood samples were obtained immediately before vaccination and after one and six months. At each time point, immunogenicity analyses by opsonophagocytic assay (OPA) and enzyme-linked immunosorbent assay (ELISA) were carried out for each of the 12 pneumococcal serotypes common for PCV13 and PPSV23 (serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) and for serotype 6A that is found only in PCV13. Specific functional antibacterial OPA titers were measured using validated OPA assays. OPA titers were defined as the serum dilution that resulted in complement-mediated killing of 50% of the assay bacteria (134-136). A standardized ELISA was used to measure the concentration of anticapsular polysaccharide IgG elicited by the vaccine. All immunogenicity analyses were performed at Pfizer's Vaccine Research Clinical Testing laboratory, Pearl River, USA. PB values, blood chemistry, immunoglobulins (protein fraction), and IgG subclass levels were analyzed locally at baseline and during follow-up.

The study's objective was to compare immune response to the two vaccines one and six months after vaccination. OPA geometric mean titers (GMTs), ELISA geometric mean concentrations (GMCs), and number of patients with a good response, were compared between the two vaccination groups. A good response was predefined as an OPA titer ≥ lower limits of quantification (LLOQ), set by the laboratory, in 8/12 common serotypes.

A power calculation using an expected difference in immune response of 20% between the two vaccines was performed to determine sample size. The estimation was based on limited data from clinical trials on CLL patients that have investigated immune responses after vaccination. Data was presented as geometric mean ratios (GMRs) with 95% confidence intervals and p-values.
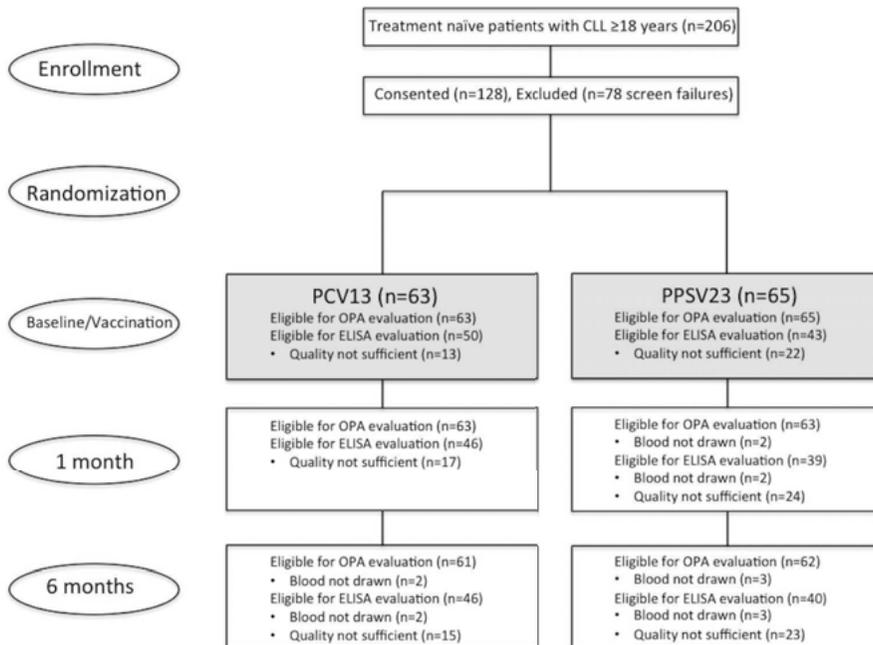
Figure 3. Study design and disposition of subjects, Paper II

Paper III

All BAL examinations performed in patients with hematological malignancies treated at the Department of Hematology, Uppsala University Hospital during the period 2004-2013, excluding those in critically ill patients in the intensive care unit, were identified retrospectively through the local bronchoscopy register and included in the study. Patients' records were reviewed for demographics, clinical characteristics, radiological findings, and test results from BAL and from "non-invasive" microbiological studies such as bacterial and fungal cultures, *Aspergillus* galactomannan (GM), 1,3-beta-D-glucan (BG) and PCR-based methods. BAL was performed by an experienced pulmonologist and a well-defined set of microbiological tests from BAL was obtained per the local standard operating procedure (SOP), including use of PSB. The SOP was updated twice during the study period with only minor changes in the set of microbiological tests (Table 3).

Changes in management of anti-infectious therapy following BAL were analyzed using test results and decisions made by the treating physicians documented in the patient records. Results from BAL sampling were considered clinically important if they altered (leading to initiation, change, or cessation) clinical management of antibacterial, antiviral, or antifungal therapy. Proce-

dure-related complications that occurred within 24 hours after the examination were recorded. The results were analyzed using descriptive methods.

Table 3. Bronchoalveolar lavage, SOP, updated 2012: recommended analyses

All hematology patients:

General culture (quantitative, aerobic, anaerobic), Fungal culture, direct fungal microscopy

Respiratory virus PCR (RS-, adeno-, para influenza-, influenzas A and B)[a]

Respiratory virus immunofluorescence (RS-, adeno-, para influenza-, influenzas A and B)[b]

Legionella PCR (cultivation is performed automatically in case of positive PCR)[c] and Legionella immunofluorescence[d]

PSB: general culture, fungal culture, Legionella PCR (culture is performed automatically in case of positive PCR)[c]

Pneumocystis jirovecii immunofluorescence (PCR is performed automatically in case of negative result)

Mycobacteria direct microscopy and general culture, General virus culture and Papanicolaou cytology

Immunocompromised patients:

CMV PCR

Herpes simplex types 1 and 2 PCR[c]

Aspergillus antigen and Aspergillus PCR[c]

In selected cases:

Mycoplasma pneumoniae, Chlamydophilia pneumoniae and pneumococci[c] PCR

M. tuberculosis complex PCR[c]

CMV antigen[b]

a Added 2012.
b Excluded 2012.
c Added 2007.
d Excluded 2007.

Paper IV

Patients with MDS, aged 18 years or older, requiring treatment with Aza (IPSS score Int-2/high-risk, chronic myelomonocytic leukemia [CMML] with 10–29% BM blasts, secondary AML [sAML] with multilineage dysplasia and BM blasts 10–29%) and with thrombocytopenia (platelet counts < 75 x 10^9/l) were enrolled in the trial from October 2011 to March 2013. Major exclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status >2, previous treatment with any TPO-R agonist, life expectancy less than three months, previous treatment for cancer other than MDS/AML within two years before the study start, and east Asian ancestry (due to different routes of metabolism of eltrombopag).

The study was designed as a phase 1, non-randomized, single-arm feasibility study. Four different doses of oral eltrombopag (50 mg, 100 mg, 200 mg, and 300 mg) were evaluated. A 3+3 patient cohorts design was used so that no new patients were started on treatment at a higher dose without confirmation of the tolerability of the previous lower dose. An independent data monitoring committee (DMC) reviewed all data from previous dose cohorts before allowing enrollment into the next cohort (Figure 4). Aza was administered in weekly cycles, 100 mg/m² subcutaneously, Monday to Friday as per local routines. Eltrombopag treatment was commenced one week before the start of Aza, and the administered orally once daily throughout three Aza cycles. One dose escalation of eltrombopag was allowed after five weeks for each patient. Patients underwent clinical and laboratory assessments during the treatment period until four weeks after discontinuation of eltrombopag. BM morphology was assessed at screening and at weeks 5 and 13, and BM for HSPC studies was obtained before treatment and prior to cycle 2.

Safety and tolerability (primary end points) parameters included clinical and laboratory Grade 3/Grade 4 non-hematological toxicities related to study medication, increase in BM and/or PB blast counts from baseline, AEs, and SAEs. Secondary endpoints included change in platelet counts per the International Working Group (IWG) criteria, efficacy of MDS treatment given as per IWG response criteria, frequency and number of units of platelet transfusions during treatment, dose delays and dose reductions of Aza, and incidence and severity of bleeding events as measured on the World Health Organization (WHO) bleeding scale.

The impact on HSPC (CD34+ CD38−) cell cycle status was analyzed with flow cytometry. After fixation and permeabilization, BM mononuclear cells were stained with surface markers CD19, CD34, CD38, CD90, and intracellular markers Ki67 and 4`, 6-diamidine-2-phenylidole dihydrochloride (DAPI) (137). Samples were analyzed on an LSRII (BD). Samples from 8 of 12 patients from screening and/or prior to cycle 2 had an adequate yield of mononuclear BM cells and were analyzed. Descriptive statistics were used. Data were expressed in median (range) or mean (standard error).

A Student t-test was used to compare platelet means at screening and at week 13.
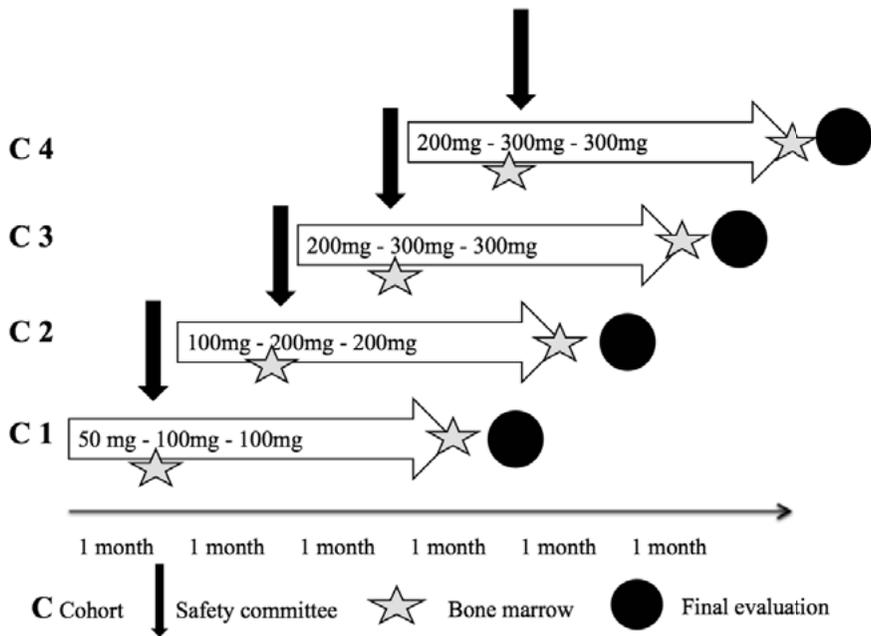

Figure 4. Study design, Paper IV

Results and Discussion

Clinical significance of serum immunoglobulin G subclass deficiency in patients with chronic lymphocytic leukemia (Paper I)

Routine analysis of IgG subclass levels in patients with CLL is probably not warranted

Hypogammaglobulinemia (particularly serum levels of IgG below normal values) is associated with an increased risk of infection in patients with CLL. The prevalence of hypogammaglobulinemia in unselected CLL patients varies between 50% to 70% in different studies (15, 30, 31, 34) and has been shown to develop over time. Patients with low IgG levels and recurrent infections can be offered prophylactic intravenous Ig substitution (14, 16, 43). IgG consists of four subclasses (IgG1, IgG2, IgG3, and IgG4), but it is not known whether pure IgG subclass deficiency (IgG subclass deficiency with total IgG, IgA, and IgM within normal range) leads to an increased risk of infectious complications. Consequently, serum levels of immunoglobulin (i.e., IgG, IgA, IgM) are routinely analyzed in clinical practice in CLL patients, whereas serum levels of subclasses of IgG usually are not (31, 37, 38).

In Paper I we studied a heterogeneous cohort of 111 CLL patients from Uppsala University Hospital. Allo SCT recipients and patients who had been referred from other hospitals were excluded. Median age at diagnosis of CLL was 67 (range 43–86) years and the median disease duration was 51 (range 4–286) months. Three out of four patients had the earliest stage of disease (Rai 0, Binet A), 36% had previously received treatment for CLL, and two patients were on regular Ig substitution as prophylaxis for infection. In accordance with other studies, in this unselected group of CLL patients, 52% had hypogammaglobulinemia, which was associated with a longer disease duration and extensive previous treatment. Notably, and in contrast to findings in several previous studies, the annual frequency of infection (including severe infections requiring hospital care and sepsis) among patients with and without hypogammaglobulinemia did not differ. Pure subclass deficiency was seen in only 5% of the study population (Table 4). The small size of this subgroup of patients made it difficult to draw definite conclusions, but no increased risk of infection was observed.

We reported a low prevalence of pure Ig-subclass deficiency together with an absence of a clear correlation between deficiency and infection—findings that do not speak in favor of routinely analyzing IgG subclasses in CLL patients. Measurement of serum levels of Ig may be justified in patients with recurrent severe infections, but routine analysis of IgG subclass levels in patients with CLL is probably not warranted. Furthermore, our findings indicate that hypogammaglobulinemia in general is, most probably, an indicator (cofactor) of advanced disease aggravated by lymphocyte toxic chemotherapy and not necessarily an independent risk factor for infectious complications.

Table 4. Prevalence of immunoglobulin deficiencies in 111 patients with CLL

Patient characteristics	n (%)
Hypogammaglobulinemia	58 (52)
Subclass deficiency	40 (36)
IgG1 deficiency	23 (21)
IgG2 deficiency	22 (20)
IgG3 deficiency	14 (13)
Pure subclass deficiency	6 (5)

Conjugated pneumococcal vaccine triggers a better immune response than polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia – a randomized study by the Swedish CLL group (Paper II)

In patients with previously untreated CLL, the efficacy of PCV13 on immune response is superior to PPSV23

Patients with CLL have increased susceptibility to infection. *Streptococcus pneumoniae* is one of the most common pathogens affecting this patient group, causing infections such as pneumonia and sepsis with sometimes even lethal outcome. Vaccination can prevent infections, but patients with CLL are known to respond poorly to the traditional polysaccharide vaccines. Conjugation of polysaccharide to protein carriers renders a thymus-dependent, memory inducing, more immunogenic vaccine. In patients with

CLL, there is no consensus on recommendation for pneumococcal vaccination, mainly because comparative studies are lacking (15, 18, 47, 53).

For Paper II we designed a randomized study to compare immune responses (measured by OPA and ELISA) to a 13-valent conjugated pneumococcal vaccine (PCV13) with responses to a conventional 23-valent capsular polysaccharide vaccine (PPSV23). 128 treatment-naïve CLL patients were vaccinated from September 2013 to June 2015. 126 and 120 patients were evaluable for immunogenicity analysis at the one- and six-month follow-ups, respectively. Median time from diagnosis to vaccination was 31 (range 1–248) months. Median age at the time of vaccination was 69 (range 46–87) years. Most patients had low stage disease on the Rai classification system and two thirds had normal IgG values prior to vaccination. Both vaccines were well tolerated.

OPA GMTs elicited by PCV13 were higher than those elicited by PPSV23 for 10/12 of the common serotypes one month after vaccination and for 5/12 of the common serotypes six months after vaccination. ELISA GMCs of serotype-specific IgG antibodies were higher in PCV13 recipients than in PPSV23 recipients for 7/12 common serotypes one month after vaccination and for 6/12 common serotypes six months after vaccination. Not surprisingly, the immune response for serotype 6A, covered by PCV13 only, was better for PCV13 than for PPSV23. PPSV23 did not trigger a better immune response than PCV13 in any serotype, regardless of analysis or time point for analysis (Table 5). A good response (defined as a response [≥LLOQ] in 8/12 common serotypes) was more common in PCV13 recipients than in PPSV23 recipients one month post vaccination (40% vs. 22%, p = 0.034) and six months' post vaccination (33% vs. 17%, p = 0.041).

OPA GMTs elicited in patients with hypogammaglobulinemia (IgG <lower normal range) were lower than those elicited in patients with normal IgG values for 11/13 serotypes one month after vaccination and for 8/13 serotypes six months after vaccination. OPA GMTs elicited in patients with a median disease duration less than 31 months were higher than those elicited in patients with a disease duration longer than 31 months (median disease duration = 31 months) in all (13/13) serotypes one month after vaccination and in 12/13 serotypes six months after vaccination. Since only two patients were high risk (III/IV) on the Rai staging system, analysis of the impact of Rai stage on vaccine response was not meaningful.

Duration of response was relatively consistent over time; for only two of the serotypes, 18C and 23 F, OPA GMTs were statistically lower at the six-month than at the one-month follow up. Measured as ELISA GMCs, only serotype 3 was lower at the six-month follow-up. For the remaining serotypes, immune responses were equal at the two time points.

We reported that a conjugated pneumococcal vaccine (PCV13) triggers a better immune response than a conventional capsular polysaccharide vaccine (PPSV23) in a majority of the pneumococcal serotypes common to both

vaccines. In the remaining serotypes, immune response was equal between the two vaccines. Negative predictive factors for vaccination response were hypogammaglobulinemia and long disease duration. PCV13 should be considered as a part of vaccination programs for CLL patients and should be administered as early as possible during the disease.

Table 5. Immunogenicity data measured as OPA GMTs (titers) and ELISA GMCs (µg/ ml), Paper II

Serotype	Analysis	Baseline		1 month				6 months				6 vs. 1 month	
		PPSV23 GM (SD)	PCV13 GM (SD)	PPSV23 GM (SD)	PCV13 GM (SD)	GMR (95% CI)	P-value	PPSV23 GM (SD)	PCV13 GM (SD)	GMR (95% CI)	P-value	GMR (95% CI)	P-value
1	OPA	5.2 (2.6)	5.8 (2.2)	10.6 (4.3)	24.0 (6.1)	2.1 (1.3-3.3)	0.002	8.8 (3.5)	14.8 (4.8)	1.6 (1.0-2.5)	0.056	0.8 (0.6-1.0)	0.095
	ELISA	0.1 (3.9)	0.1 (3.2)	0.3 (6.1)	0.4 (4.6)	1.4 (0.9-2.1)	0.197	0.3 (6.3)	0.4 (4.6)	1.2 (0.7-1.8)	0.520	0.9 (0.7-1.1)	0.236
3	OPA	5.4 (2.2)	5.5 (2.2)	9.7 (3.6)	15.5 (4.0)	1.6 (1.1-2.2)	0.010	7.5 (2.8)	10.7 (3.9)	1.4 (1.0-2.0)	0.052	0.9 (0.7-1.1)	0.328
	ELISA	0.2 (4.1)	0.2 (3.5)	0.3 (4.3)	0.3 (4.0)	1.3 (0.9-1.8)	0.193	0.3 (4.9)	0.2 (4.1)	1.0 (0.7-1.4)	0.867	0.7 (0.6-0.9)	0.013
4	OPA	6.3 (3.5)	6.6 (4.1)	27.4 (10.3)	106.3 (16.6)	3.5 (1.6- 7.5)	0.002	19.9 (8.8)	52.7 (13.0)	2.5 (1.2- 5.4)	0.019	0.7 (0.5-1.2)	0.224
	ELISA	0.1 (3.0)	0.1 (4.4)	0.2 (5.4)	0.4 (5.4)	1.7 (1.1-2.5)	0.015	0.2 (5.5)	0.4 (4.6)	1.7 (1.1-2.5)	0.010	1.0 (0.8-1.3)	0.921
5	OPA	5.0 (1.8)	5.3 (2.3)	9.8 (3.4)	18.1 (7.4)	1.8 (1.1-2.7)	0.012	8.6 (3.0)	13.2 (5.7)	1.4 (0.9-2.2)	0.116	0.8 (0.6-1.1)	0.136
	ELISA	1.2 (3.3)	1.2 (3.0)	1.8 (2.9)	1.8 (3.8)	1.2 (0.9-1.6)	0.135	1.6 (3.2)	1.8 (3.7)	1.3 (1.0-1.6)	0.108	1.0 (0.8-1.2)	0.866
6A	OPA	7.6 (4.4)	6.6 (3.9)	15.3 (7.1)	78.2 (13.1)	5.3 (2.8-10.1)	<0.001	15.4 (7.0)	65.2 (13.2)	4.4 (2.3- 8.4)	<0.001	0.8 (0.6-1.1)	0.170
	ELISA	1.2 (3.6)	0.9 (3.1)	1.3 (2.8)	1.5 (3.7)	1.6 (1.2-2.1)	0.004	1.3 (3.0)	1.5 (4.4)	1.5 (1.1-2.0)	0.009	0.9 (0.8-1.1)	0.295
6B	OPA	13.4 (7.3)	13.3 (7.1)	62.6 (12.3)	100.3 (15.4)	1.2 (0.6- 2.5)	0.572	49.6 (10.4)	74.3 (16.1)	1.4 (0.6- 2.8)	0.421	1.2 (0.8-1.9)	0.333
	ELISA	0.7 (4.7)	0.7 (3.5)	1.1 (4.4)	1.3 (4.3)	1.3 (0.9-1.8)	0.157	1.2 (4.4)	1.2 (5.2)	1.2 (0.9-1.7)	0.241	1.0 (0.8-1.2)	0.689
7F	OPA	12.0 (5.4)	10.2 (5.5)	47.9 (10.0)	83.8 (12.1)	2.1 (1.1-3.9)	0.025	34.7 (8.5)	75.4 (11.6)	2.4 (1.3- 4.6)	0.008	1.0 (0.8-1.4)	0.787
	ELISA	0.5 (4.0)	0.4 (4.4)	1.0 (5.3)	1.3 (6.9)	1.6 (1.1-2.5)	0.020	1.0 (5.8)	1.1 (7.0)	1.4 (1.0-2.2)	0.085	0.9 (0.7-1.1)	0.217
9V	OPA	12.6 (7.0)	17.9 (8.9)	29.9 (10.1)	111.2 (16.7)	2.9 (1.4-6.1)	0.005	27.1 (8.7)	62.9 (15.2)	1.9 (0.9- 4.0)	0.084	0.7 (0.4-1.1)	0.087
	ELISA	0.6 (4.8)	0.5 (3.8)	0.9 (4.7)	1.4 (6.2)	1.9 (1.3-2.7)	0.001	0.9 (5.2)	1.1 (5.8)	1.6 (1.1-2.3)	0.011	0.9 (0.7-1.0)	0.106
14	OPA	66.5 (10.4)	82.2 (10.4)	207.8 (11.7)	231.3 (9.5)	0.9 (0.5- 1.7)	0.750	180.0 (9.2)	180.4 (9.4)	0.9 (0.5- 1.7)	0.838	1.0 (0.7-1.4)	0.970
	ELISA	1.0 (4.4)	0.9 (5.1)	2.3 (6.7)	1.8 (7.2)	0.9 (0.7-1.3)	0.681	2.4 (5.9)	1.9 (6.1)	0.9 (0.7-1.3)	0.620	0.9 (0.8-1.1)	0.529
18C	OPA	19.9 (6.8)	21.4 (7.1)	63.5 (9.7)	253.2 (9.3)	3.9 (2.1- 7.3)	<0.001	51.0 (9.1)	128.6 (8.2)	2.8 (1.5- 5.2)	0.001	0.7 (0.5-0.9)	0.015
	ELISA	0.7 (3.5)	0.7 (4.3)	1.2 (4.0)	2.1 (6.0)	1.7 (1.2-2.4)	0.004	1.3 (4.2)	1.7 (5.3)	1.5 (1.0-2.1)	0.036	0.9 (0.7-1.0)	0.060
19A	OPA	17.1 (4.6)	18.9 (5.1)	39.7 (6.2)	80.5 (8.6)	1.9 (1.2-3.0)	0.010	37.9 (6.4)	62.0 (7.8)	1.6 (1.0- 2.5)	0.068	0.8 (0.6-1.2)	0.297
	ELISA	2.2 (3.4)	2.0 (3.5)	2.8 (3.4)	3.5 (4.4)	1.6 (1.3-2.1)	<0.001	2.7 (3.9)	3.3 (4.7)	1.4 (1.1-1.9)	0.005	0.9 (0.7-1.0)	0.130
19F	OPA	12.1 (5.0)	11.5 (4.6)	36.4 (10.2)	59.2 (11.4)	2.0 (1.1-3.6)	0.016	29.9 (8.7)	37.1 (9.1)	1.7 (1.0- 2.9)	0.075	0.9 (0.6-1.3)	0.473
	ELISA	0.6 (6.1)	0.7 (3.5)	0.7 (6.1)	1.5 (5.5)	1.8 (1.3-2.5)	0.001	0.7 (6.3)	1.3 (5.2)	1.5 (1.2-2.1)	0.023	0.8 (0.7-1.1)	0.131
23F	OPA	8.0 (3.7)	5.9 (2.8)	12.0 (6.1)	33.2 (11.1)	3.8 (2.1- 6.9)	<0.001	12.9 (5.8)	20.5 (8.9)	2.3 (1.3- 4.2)	0.006	0.6 (0.4-0.9)	0.012
	ELISA	0.8 (3.9)	0.7 (3.6)	1.2 (4.1)	1.4 (5.5)	1.5 (1.1-2.2)	0.018	1.2 (4.2)	1.5 (5.4)	1.5 (1.1-2.2)	0.020	0.9 (0.8-1.1)	0.508

Utility of Broncho Alveolar Lavage in Diagnosing Respiratory Tract Infections in Patients with Hematological Malignancies: Are Invasive Diagnostics Still Needed? (Paper III)

BAL is still a valuable method for diagnosing respiratory tract infections in patients with hematological malignances

Patients treated for hematological malignancies have an increased risk of serious infection, which makes diagnosis, and prompt initiation of anti-microbial therapy essential. The use of BAL as a means of identifying the cause of pulmonary infiltrates is widespread, and a standardized diagnostic approach to microbiological sampling is usually applied. However, non-invasive diagnostic methods are under constant improvement, which makes it essential to re-evaluate established ones (70-73).

In Paper III, we retrospectively reviewed BAL examinations performed in patients with hematological malignances at Uppsala University Hospital over a 10-year period. A relatively extensive set of microbiological tests from BAL had been used per local SOP.

In 59 (39%) of 151 BAL cases a microbiological cause of the infectious episode was identified, and in 38 (25%), findings from BAL had an impact on clinical management either by establishing or contributing to the final diagnosis. In six cases (4%) negative findings from BAL facilitated cessation of initiated microbiological therapy. The most common diagnoses in our study population were invasive pulmonary aspergillosis (IPA) and *Pneumocystis jirovecii* pneumonia (PJP). Results are summarized in Table 6. IPA diagnosis was based on results from BAL in 65% and PJP diagnosis in 93%, respectively. Several investigations analyzed per local SOP, such as PCR for *Legionella* spp. and *Clamydophilia pneumoniae* and samples obtained using PSB rendered no positive results. Complications were few and mainly mild.

We concluded that BAL still contributes to either verifying or excluding some important respiratory tract infections in patients with hematological malignancies, particularly IPA and PJP. However, the development of non-invasive methods may reduce the need to perform BAL in the future. Standardized procedures for BAL sampling should be continuously revised to avoid unnecessary microbiological tests.

Table 6. Diagnosis and impact of BAL on clinical management

Impact of BAL on clinical management	n = 151 (%)
Diagnosis based solely on BAL/Diagnosis based on BAL and other diagnostic methods/Diagnosis based on methods other than BAL/Negative findings from BAL leading to cessation of initiated therapy/No impact	27/11/21/6/107 (18/7/14/4/71)
Final diagnosis*	n=157 (%)
Aspergillus pneumonia/Pneumocystis pneumonia/Other specified cause†/Pneumonia with unknown cause/Neutropenic fever and/or respiratory tract symptoms with unknown cause	23/14/26/85/9 (15/9/17/54/6)

*More than one diagnosis in some cases
† Bacterial pneumonia with specified cause, invasive candidosis, mycobacterium tuberculosis, RS-virus, CMV-pneumonitis, pulmonary graft versus host disease (GVHD), bacterial sinusitis with established cause, drug reaction, heart failure, Legionella pneumonia, rhino virus, pulmonary embolism, Cryptogenic organizing pneumonia (COP)

A pilot phase I dose-finding safety study of the thrombopoietinreceptor agonist, eltrombopag, in patients with myelodysplastic syndrome treated with azacitidine (Paper IV)

Eltrombopag in combination with azacitidine in high-risk MDS patients with thrombocytopenia is feasible and well tolerated

Thrombocytopenia is an independent adverse prognostic factor in patients with MDS (105). The hypomethylating agent azacitidine is first-line treatment for most patients with high-risk disease. It prolongs overall survival, reduces the risk of leukemic transformation, and improves quality of life (98-100). It is, however, associated with aggravated thrombocytopenia, at least during initial treatment. Eltrombopag is an orally administered small-molecule thrombopoietin-receptor (TPO-R) agonist approved for the treatment of thrombocytopenia in patients with ITP (110). It has also been shown to inhibit proliferation of leukemia cell lines *in vitro* (128, 129, 131, 132).

For Paper IV we designed a phase I clinical trial to explore the safety and tolerability of eltrombopag administered in combination with azacitidin to patients with MDS. Twelve high-risk MDS patients were enrolled and received eltrombopag in dose-escalation cohorts (50, 100, 200, and 300 mg) during three cycles of azacitidine. Patients receiving eltrombopag in doses up to 200 mg daily had no SAEs related to the study medication; all reported events were related to the underlying hematological disease and/or Aza

treatment. Upon dose escalation of eltrombopag to 300 mg in the fourth cohort, two dose-limiting toxicities (DLTs) were observed: deep vein thrombosis (DVT) and intractable skin rash. Although neither of these DLTs could be related with certainty to the study medication, an eltrombopag dose of 200 mg was considered the maximum tolerable dose (MTD). No cataract formation, BM fibrosis, or increase in PB or BM blasts from base line, were seen during the study period. Bleeding manifestations were mild and uncommon. Notably, the case of DVT was observed in a patient with progressive, refractory sAML, whereas no thromboembolic complications were seen in the other patients, including three with previous histories of DVT.

While efficacy was not a primary endpoint in the current study, we reported findings that suggest a possible anti-tumor activity as well as an effect on alleviating thrombocytopenia in a cohort of high-risk MDS patients treated with Aza. Among the nine patients who concluded the study as planned, one had CR and three patients obtained BM CRs. Platelet response was reported in four patients, whereas stable platelet counts were seen in four other patients. No patient had progression of thrombocytopenia despite ongoing treatment with Aza. The mean platelet counts at screening and at the end of the third Aza cycle were 36 (SD 16) and 94 (SD 85) x 10^9/L respectively (p = 0.09) (Figure 5). Dose delays in Aza were related to infectious complications and skin rash, but were in no case related to thrombocytopenia. We also reported the novel *ex vivo* finding that in this patient group, there was no increase in the cycling of HSPCs, progenitor cells, or strictly defined HSCs. This strengthens previous data from *in vitro* studies suggesting that eltrombopag does not have a stimulatory effect on primitive BM cells (128).

We concluded that eltrombopag up to a dose of 200 mg daily, in combination with Aza in high-risk MDS patients with thrombocytopenia, is feasible and well tolerated. The design of the randomized, placebo-controlled phase III study SUPPORT, powered to detect improvements in platelet count, prevention of major bleeding, and the possibility of an additive anti-leukemic effect of eltrombopag on high-risk MDS patients, was based on findings from our study.
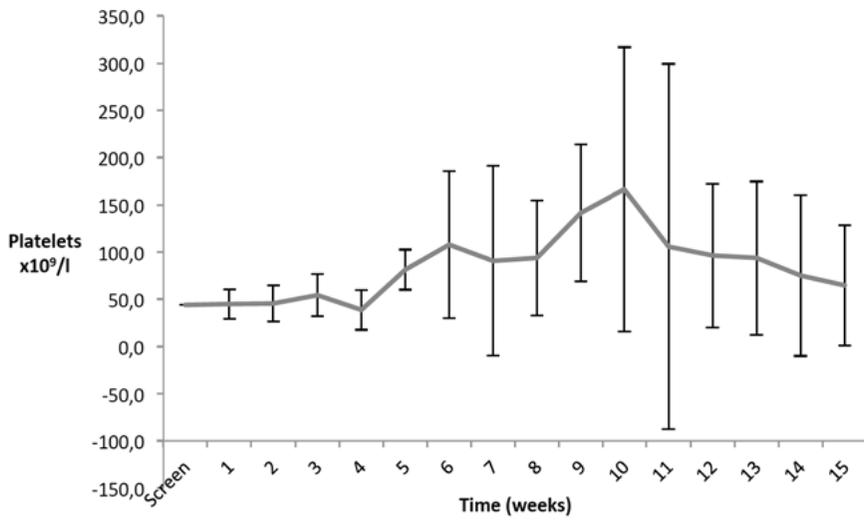
Figure 5. Platelet counts (mean/SD) at screening and at weeks 1 to 15 for nine patients with MDS receiving eltrombopag and Aza

Conclusions and Future Perspectives

The results of this thesis may be summarized as follows:

- IgG subclass deficiency in CLL patients with total levels of IgG, IgA, and IgM within normal range is rare. Our results do not indicate that these patients have an increased risk of infection that would make prophylactic intravenous gamma globulin substitution an appropriate intervention. Routine analysis of IgG subclasses in patients with CLL is therefore not warranted.
- The combination of the TPO-R agonist eltrombopag with the hypomethylating agent Aza in high-risk MDS patients with thrombocytopenia is feasible and well tolerated in doses up to 200 mg eltrombopag daily. A potential for alleviating thrombocytopenia by adding eltrombopag to these patients' medications cannot be ruled out.
- BAL contributes to either verifying or excluding some important respiratory tract infections, particularly IPA and PJP, in patients with hematological malignancies. Standardized procedures for BAL sampling should be continuously revised to avoid superfluous microbiological tests.
- In patients with previously untreated CLL, the efficacy of PCV13 on immune response is superior or equal to PPSV23 for all serotypes common for the two vaccines. PCV13 should therefore be considered part of vaccination programs against *Streptococcus pneumoniae* for these patients and should be administered as early as possible in the disease.

We live in an era of rapidly changing treatment and diagnosis of hematological malignancies. Particularly for B-cell malignancies such as CLL, a range of targeted agents was introduced in the clinic during the writing of this thesis. However, although many of these new agents show better efficacy in tumor response than the older ones, they still have side effects such as increased risks of infectious and bleeding complications. This is a challenge for hematologists and emphasizes the need for optimized supportive care to reach the ultimate goal of improved patient outcomes. Methods to diagnose, prevent, and alleviate infections and bleeding should be under constant development to reach this goal. It is also important to challenge established methods to make room for new and better ones. Several questions that warrant further studies have arisen during the writing of my thesis.

Response to vaccinations is known to abate over time. A follow-up study on Paper II to see how immunogenicity in responders to vaccination develops over time would be feasible and important and might help to answer questions about suitable time points for, and the potential effects of, revaccination. The strategy of combining PCV13 with subsequent administration of PPSV23 to broaden the immune response through coverage of additional pneumococcal serotypes is generally recommended (12). The efficacy of the combination is, however, poorly studied and would also warrant a randomized study.

A plethora of retrospective studies evaluate the diagnostic yield of BAL in different patient populations. Such studies, of course, have several limitations. The complex nature of the infections affecting these patients and the high degree of subjectivity in establishing a diagnosis make estimates of the diagnostic value difficult. Different ways of measuring diagnostic yield and differences in patient selection also make comparisons between studies near impossible. To establish the diagnostic yield of BAL in patients with hematological malignancies and pulmonary infiltrates, a prospective study comparing BAL with modern non-invasive infectious diagnostics is highly warranted.

Based on the safety and tolerability data from Paper IV, the platelet-supportive effects of eltrombopag in high-risk MDS patients receiving Aza were investigated in the randomized, placebo-controlled (n = 356), multi-center phase III SUPPORT trial. Patient enrollment was completed in April 2016 and preliminary analyses were presented by Dickinson et al at the American Society of Hematology (ASH) congress in December 2016. In contrast to our preliminary findings (Paper IV), eltrombopag given concomitantly with Aza was found to be inferior to placebo/Aza in alleviating thrombocytopenia (138), data that clearly do not speak in favor of this particular treatment combination. However, previous studies have shown that eltrombopag as a single agent can alleviate thrombocytopenia and bleeding in patients with high-risk MDS and AML without, apparently, increasing the risk for leukemic transformation. Hence, eltrombopag may be a useful addition in the palliative care therapy arsenal for selected cases in this group of patients with a short estimated survival (121). Eltrombopag has also shown efficacy in low-risk MDS patients (120), although its therapeutic use is probably limited; thrombocytopeni in these patients are not usually severe and therefore usually not associated with bleeding.

Svensk populärvetenskaplig sammanfattning

Blodcancer är en brokig sjukdomsgrupp med ursprung i benmärgen, kroppens blodbildande organ. Brist på röda och vita blodkroppar respektive blodplättar är vanligt vid blodcancer och leder till trötthet, infektionskänslighet och risk för blödningar. Den ökade infektionsbenägenheten hos blodcancerpatienter beror på brist på vita blodkroppar som också fungerar sämre än normalt, men kan även vara en bieffekt av behandling. På motsvarande sätt kan en sjukdomsorsakad brist på blodplättar (trombocytopeni), förvärras efter behandling, både på kort och lång sikt.

En bidragande orsak till ökad infektionskänslighet vid vissa blodsjukdomar är hypogammaglobulinemi (antikroppsbrist). Immunoglobuliner kan ges intravenöst till patienter med hypogammaglobulinemi och minska infektionsrisken. Pneumokocker kan orsaka allvarliga infektioner. Det finns två typer av vaccin mot denna bakterie, dels det äldre kapselvaccinet där immunförsvaret aktiveras genom att reagera mot den kapsel som omger bakterien och dels ett s.k. konjugatvaccin där ett protein binds till bakteriekapseln för att förstärka immunsvaret. En metod för att diagnosticera en infektion i luftvägarna är bronkoalveolärt lavage (BAL), vilket innebär att man med hjälp av ett särskilt instrument (bronkoskop) kan ta prover från luftrören.

Det övergripande syftet med detta doktorandprojekt var att förbättra möjligheten att upptäcka och förhindra infektioner och blödningar hos blodcancerpatienter. Avhandlingen består av följande fyra delprojekt:

Hypogammaglobulinemi drabbar ofta patienter med Kronisk lymfatisk leukemi (KLL). I det första projektet undersökte vi hur vanlig det är med brist på någon av de fyra undergrupperna av immunoglobulin G (IgG) vid KLL och om sådan brist ökar risken för infektioner. Undergrupper av IgG i blodet analyserades på 146 patienter med KLL och deras journaler undersöktes i syfte att ta reda på i vilken utsträckning de drabbats av infektioner. Brist på undergrupper av IgG visade sig vara ovanligt och troligen inte medföra en ökad infektionsrisk. Sannolikt är det därför onödigt att rutinmässigt analysera undergrupper av IgG på KLL-patienter.

I det andra delprojektet lottades 128 patienter KLL till vaccination mot pneumokocker med ett kapselvaccin eller ett konjugatvaccin. Syftet var att undersöka vilket av de båda vaccinen som ger upphov till bäst immunsvar hos dessa patienter. Genom att mäta immunsvaret i blodprover från patienterna efter det att de vaccinerats, kunde vi konstatera att hos KLL-patienter är konjugatvaccinet, i likhet med vad fallet är för barn och flera andra pati-

entgrupper, mer effektivt än kapselvaccinet. Lång varaktighet av sjukdomen och hypogammaglobulinemi var faktorer som medförde ett sämre immunsvar. Vi drog slutsatsen att patienter med KLL bör vaccineras med ett konjugatvaccin mot pneumokocker så snart som möjligt efter att diagnosen ställts.

I det tredje delprojektet undersöktes hur effektiv BAL är för att hitta den bakomliggande orsaken till en infektion i luftvägarna hos blodcancerpatienter. Vid en journalgenomgång av 151 BAL-undersökningar gjorda under en tioårsperiod på Akademiska sjukhuset, där provtagning för infektionsdiagnostik utförts enligt ett standardiserat förfarande, kunde en sådan orsak identifierades i 39 % av fallen. I 25 % av fallen bidrog BAL till att ställa diagnos och i 4 % av fallen möjliggjorde negativa fynd från BAL att påbörjad infektionsbehandling kunde avslutas. De vanligaste orsakerna till lunginfektionen var *Aspergillus* spp. (mögelsvamp) och *Pneumocystis jirovesii* där BAL bidrog till att ställa diagnos i flertalet av fallen. Vi kunde konstatera att många analyser som utförts enligt det standardiserade förfarandet var onödiga, då de aldrig gav några positiva resultat.

Patienter med den i många fall mycket allvarliga sjukdomen myelodysplastiskt syndrom (MDS) kan behandlas med läkemedlet azacitidine där målet är symtomlindring och förlängd överlevnad. Trombocytopeni är vanligt hos MDS-patienter och den kan förvärras under azacitidinebehandlingen. Läkemedlet eltrombopag ökar bildningen av trombocyter i benmärgen, men är hittills inte godkänt för behandling av MDS. I det sista delprojektet behandlades 12 patienter med MDS samtidigt med azacitidine och eltrombopag med huvudsyfte att undersöka hur patienterna tål denna läkemedelskombination. Dosen av eltrombopag höjdes succesivt under studiens gång för att finna den maximalt tolererade dosen. Vi kunde konstatera att kombinationsbehandlingen med eltrombopag i doser upp till 200 mg/dag tolereras väl, men att doser högre än 200 mg per dag inte kan rekommenderas på grund av ökad risk för biverkningar. Även om syftet med studien inte var att mäta effekt i form av ökade trombocytnivåer och minskade blödningskomplikationer, så kunde vi notera detta hos flera av patienterna.

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