Neutropenia in cancer patients, risk prediction models of neutropenia, and supportive measures

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Alena Maria Pfeil

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auf Antrag von

Prof. Dr. Christoph R. Meier (Fakultätsverantwortlicher)

Prof. Dr. Thomas D. Szucs und PD Dr. Matthias Schwenkglenks (Dissertationsleiter)

PD Dr. Margot Mütsch (Ko-Referentin)

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Prof. Dr. Jörg Schibler

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Dedicated to my loved ones

Table of contents

List of tables

List of figures

List of abbreviations

AC	Doxorubicin, cyclophosphamide
Adj.	Adjusted
AE	Adverse event
ALT	Alanine aminotransferase
AML	Acute myeloid leukaemia
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body mass index
BSA	Body surface area
CCI	Charlson comorbidity weighted index
CIN	Chemotherapy-induced neutropenia
CIRS	Cumulative Illness Rating Scale
CLL	Chronic lymphocytic leukaemia
Cong.	Congress
COPD	Chronic obstructive pulmonary disease
CPRD	Clinical Practice Research Datalink
CTCAE	Common Terminology Criteria for Adverse Events
DAG	Directed acyclic graph
DLBCL	Diffuse large B-cell lymphoma
EORTC	European Organization for Research and Treatment of Cancer
ER	Estrogen receptor
FDR	False discovery rate
FEC	5-Fluorouracil, epirubicin, cyclophosphamide
FLIPI	Follicular Lymphoma International Prognostic Index
FN	Febrile neutropenia
FOIL	5-Fluorouracil, leucovorin, oxaliplatin, irinotecan
FOLFIRI	5-Fluorouracil, leucovorin, irinotecan
FOLFOX	5-Fluorouracil, leucovorin, oxaliplatin
G-CSF	Granulocyte colony-stimulating factor
GEC	Gastro-esophageal cancer
GP	General practitioner
GPRD	General Practice Research Database
HB	Haemoglobin
HER2	Human epidermal growth factor receptor 2
ICD	International Classification of Diseases, Ninth Revision, Clinical Modification
INC-EU	Impact of Neutropenia in Chemotherapy-European Study Group
IQR	Interquartile range
IR	Incidence rate
IRR	Incidence rate ratio
ISAC	Independent Scientific Advisory Committee
iv	Intravenous

HR	Hazard ratio
MHRA	Medicines and Healthcare Products Regulatory Agency
NA	Not applicable
(N)HL	(Non-)Hodgkin lymphoma
NIHR	National Institute for Health Research
NHS	National Health Service
NOS	Not otherwise specified
NPV	Negative predictive value
NS	Not statistically significant
NSCLC	Non-small cell lung cancer
OR	Odds ratio
OS	Overall survival
PP	Primary prophylaxis
PPV	Positive predictive value
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone
RCT	Randomised controlled trial
RDI	Relative dose intensity
ROC	Receiver operating characteristic
RR	Relative risk
SD	Standard deviation
S-HAM	High dose cytosine arabinoside and mitoxantrone
SN	Severe neutropenia
SNP	Single nucleotide polymorphism
SP	Secondary prophylaxis
TC	Docetaxel, cyclophosphamide
TCF	Docetaxel, cisplatin, l-folinic acid, 5-fluorouracil
TNM	Tumour size, nodes, metastasis
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
yrs	Years

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Preface

Febrile neutropenia in cancer patients can be fatal. Can we predict which patients are at high risk of febrile neutropenia and target prophylactic measures to those patients? To answer this question, it is essential to know the incidence of neutropenia in cancer patients, to identify risk factors of febrile neutropenia and effective prophylactic measures. The aim of this work was to describe the epidemiology of neutropenia and febrile neutropenia including risk factors, to develop and externally validate a risk prediction model for febrile neutropenia, and to summarise the efficacy of prophylactic measures for neutropenia. This work is based on four published studies and the thesis is structured as follows.

First, a general and more specific introduction to the research addressed in this work is provided. The general introduction deals with a short description of epidemiology and its basic principles and terminology including epidemiologic study designs (1.1). This is complemented by a more specific introduction that provides an overview of cancer and neutropenia including a short summary for breast cancer, chronic lymphocytic leukaemia (CLL) and neutropenia (1.2). A brief section following the introduction describes the overall objectives of the thesis (2).

In the methods' section, the Poisson regression used to identify trends in incidence is explained (3.2.2). Other regression analyses include logistic regression (3.2.1) to determine risk factors and to develop a risk prediction model and Cox proportional hazard regression (3.2.3) to analyse survival data. The approach for a systematic review is briefly described (3.3).

In a separate results' section (4), the methodological details and results of the four peer-reviewed publications this work is based on are reproduced. Finally, the findings of the studies are discussed in a broader context and potential future directions are presented (5), and conclusions are drawn (6). A comprehensive reference list is provided (7). Supplementary material is available in the Appendix (8).

Summary

Epidemiology studies the causes and distribution of population health and disease conditions in defined populations. It identifies risk factors for disease which may help to prevent disease and promote health.

Each year, the American Cancer Society describes the epidemiology of cancer in the USA. Breast cancer and CLL are the most common cancers in women and adults, respectively. European data for CLL are limited. For both cancers, chemotherapy is an important treatment option. But side effects such as neutropenia and infections remain the principal dose-limiting toxicities, which may affect the effectiveness of cancer chemotherapy. Several studies evaluated risk factors for chemotherapy-induced neutropenia (CIN; absolute neutrophil count [ANC] $<1.5x10^9$/L) and febrile neutropenia (FN; ANC $<0.5x10^9$/L and oral temperature ≥38° for more than 1 hour): e.g. older age, recent infection, prior chemotherapy, and planned relative dose intensity greater than 85% of standard chemotherapy dosing. The prophylactic use of granulocyte colony-stimulating factors (G-CSFs) has been shown to be protective.

Based on the above mentioned risk factors, a number of risk prediction models have been developed over the years. Very often, the risk prediction models considered patient-related, tumour-related, treatment-related, or genetic factors. The majority of these models are not validated using an independent dataset. Systematic reviews of G-CSFs to prevent neutropenia are available, but do not include new long-acting G-CSFs or observational study designs.

To address the epidemiology of CLL, the incidence and risk factors of CIN and FN, and to develop and externally validate a risk prediction model for the occurrence of FN including a broad range of risk factors, three quantitative studies were conducted and published. The fourth published study summarised the efficacy, effectiveness and safety of G-CSFs for the prevention of CIN and FN.

For the first study, the author conducted a cohort analysis of the UK Clinical Practice Research Datalink (CPRD) to identify the epidemiology of CLL, the incidence of neutropenia, and changes in medical resource utilisation of CLL patients. Due to limited data regarding the incidence of neutropenia, the study focused on the epidemiology of CLL and medical resource utilisation of CLL patients. The incidence of CLL was 6.2 per 100’000 person-years and remained stable between 2006 and 2011. Medical resource utilisation in

CLL patients increased over the time period from 2000 to 2012. Primary care data from the UK CPRD seemed to be valid to determine the incidence of CLL. These data may not reflect the total of medical resource use in CLL patients as chemotherapy and treatment of related complications such as infections and neutropenia are mainly performed in secondary or tertiary care.

The second study addressed the identification of risk factors and the development of a risk prediction model for FN in a hospital-based breast cancer cohort. Risk factors for FN were lower platelet count and haemoglobin, higher alanine aminotransferase (ALT), and specific allele variants of two single nucleotide polymorphisms (SNPs) in a gene involved in multidrug resistance. Genetic testing beforehand might be helpful to identify patients at a very high risk of FN. Predictive performance of the model was improved by adding genetic information but overall remained limited.

The third study used an available risk prediction model for FN in Non-Hodgkin lymphoma (NHL) patients and applied its prediction rules to an independent dataset of NHL patients. Age, weight, baseline white blood cell count, and planned chemotherapy dose were confirmed to predict the risk of FN. However, there was a decrease of the predictive performance in the independent validation dataset. This limits its use in clinical practice. But if successful risk prediction models are developed and externally validated, these may help to optimally target prophylaxis with G-CSFs to those patients at high risk of FN.

Finally, a systematic literature review was conducted to identify studies evaluating the efficacy, effectiveness and safety of G-CSFs in the prevention of CIN and FN. Most studies showed better efficacy and effectiveness for the long-acting pegfilgrastim than daily filgrastim. Efficacy and safety profiles of new long-acting G-CSFs such as lipegfilgrastim and balugrastim were comparable to pegfilgrastim. In times of increasing health care costs and scarce resources, the cost-efficient use of supportive measures is necessary.

The studies this work is based on showed that the availability of and access to appropriate data sources are necessary to develop and systematically validate risk prediction models. The findings contribute to the development of an evidence-based, efficient and cost-efficient approach to prevent neutropenia in cancer patients.

1 Introduction

1.1 Epidemiology

The word epidemiology originates from the Greek words “epi”, meaning among or on; “demos”, meaning population; and “logos” meaning study or discourse [1]. It is defined as the study of the distribution and determinants or risk factors of disease in a defined human population [2]. Epidemiology is the basic science of public health and may help to prevent disease and promote health of the population.

Epidemiological studies were already conducted before the 19th century. Principles that still apply to good epidemiologic work have been manifested by the world’s first epidemiologist John Graunt (1620-1674) [3]. His first and only published work included several methodological aspects of epidemiology [4]. A prominent example of another epidemiological study is John Snow’s (1813-1858) study during cholera epidemics in London [2]. The physician demonstrated that only those who drank infected water from a specific water pump contracted the disease. Without knowing the disease-causing pathogen, he demonstrated the mode of transmission of cholera.

In high-income countries, most infectious diseases were controllable after the introduction of hygiene measures, vaccinations and antibiotics, whereas non-communicable diseases such as cardiovascular disease and cancer became a major health burden [2]. To gain knowledge in non-infectious diseases, the USA initiated many population-based studies such as The Framingham Heart Study [5], the water fluoridation study [6] or the Smoking and Health study [7], where basic epidemiological principles were applied. A very famous and classic epidemiological study was published in 1950 by Sir Richard Doll and Sir Austin Bradford Hill [8]. They conducted a case-control study (see section 1.1.2.1 for a definition) that demonstrated a link between tobacco smoking and lung cancer.

Important basic concepts of epidemiology were defined later. A pioneering work was published by Sir Austin Bradford Hill in 1965 defining criteria to separate causal from non-causal explanations [9]. His published criteria were an expansion of the former US Surgeon General’s report criteria [7] and are described in the next section. Other statisticians that contributed significantly to the development of modern epidemiology were Jerome Cornfield, Nathan Mantel, Norman Breslow and Ross L. Prentice [10]. Many of their epidemiological concepts are still popular.

1.1.1 Basic concepts and terminology

Three types of epidemiology can be distinguished; descriptive, analytical and interventional epidemiology [11]. Descriptive epidemiology aims to identify and classify disease entities and to describe the natural history of disease including transmission, distribution, and evolution of the disease. Usually, descriptive epidemiology does not generate hypotheses. In analytical epidemiology, a case group and a control group need to be defined to identify determinants and potential causes of the disease, to define risk factors, and to measure the level of risk. Interventional epidemiology defines health problems, and designs and measures the impact of solutions.

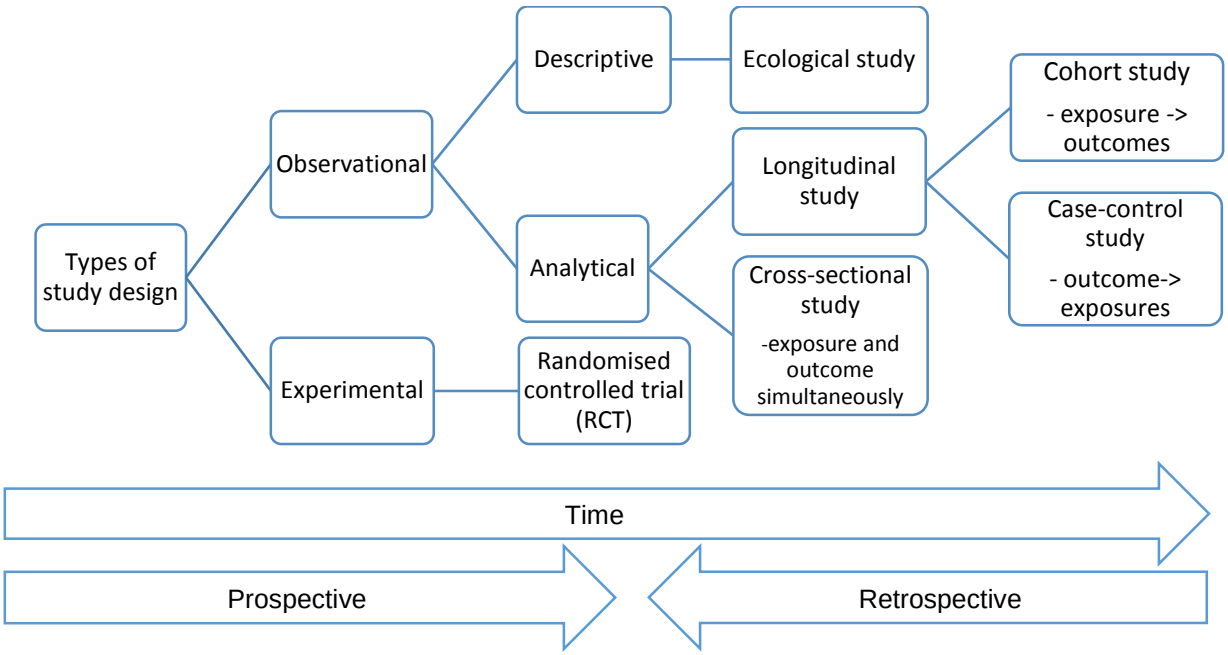
The concept of analytical epidemiology is to describe an association between an exposure (e.g. smoking) and an outcome (e.g. lung cancer) or to describe causality [11]. Hill's criteria for assessing causality are: the strength of association, i.e. how much higher is the risk of an outcome in the exposed compared to the unexposed group; consistency which means others have observed the same association; specificity in the sense of the association or the magnitude of the association; temporality meaning that the exposure precedes the outcome; a biological gradient, i.e. dose-response relationship; plausibility in terms of biologically plausible; coherence meaning that it does not interfere with the natural or biologic history of the disease; an experiment meaning that the avoidance of an exposure leads to less outcomes; and analogy with other exposure-outcome relationships [9]. Nowadays, in the context of multifactorial disease and research questions, the assessment of causality is more complex. Hill's criteria may no longer be appropriate for assessing causality [4,12].

However, today's definition of causality contains Hill's concept of temporality. Causality is defined as the relationship between an event and a second event whereby the second event is a consequence of the first, i.e. the exposure must precede the outcome [13,14]. If there is an interest in the effect of a particular exposure, the effect is measured in an exposed population and the difference to the effect which would have been observed in the same, but non-exposed population is the effect due to the exposure we are interested in (counterfactual model) [15]. Confounding and bias (discussed in section 1.1.3), but also chance and reverse causality can provide alternative explanations for the observed differences in effect between the exposed and non-exposed groups.

1.1.2 Types of epidemiologic studies

Based on the three types of epidemiology, descriptive, analytical and interventional epidemiology, six different types of studies can be distinguished (Figure 1.1-1) [11].

Figure 1.1-1: Most common types of epidemiological studies



1.1.2.1 Cohort and case-control studies

Conducting a cohort study, we consider one exposure (risk or protective factor) and observe if one or more outcomes of interest (e.g. disease or not) occur in the exposed (with risk factor) or non-exposed (without risk factor) groups which are followed over time [16]. The non-exposed group should be as similar as possible to the exposed group, except for the exposure of interest.

In case-control studies, the outcome is given (e.g. cases are defined as having the disease and controls are defined as being disease-free) [17]. The frequency of one or more exposures in subjects with or without the outcome is assessed. Selection of the appropriate control group is crucial. In nested case-control studies, cases are identified during the cohort study.

Cohort studies can be prospective, meaning that the population is observed forward in time and cases have not yet occurred, or retrospective, meaning that cases have already occurred and the study looks back in time as in case-control studies [18,19]. Retrospective studies are faster and cheaper than prospective studies, but the availability of exposure data can be problematic. Despite the additional effort compared to retrospective studies,

prospective studies are more frequent. The quality of data and the complete measurement of exposure can be ensured in prospective studies.

1.1.2.2 Other study designs

Randomised controlled trials (RCTs) are studies in which the efficacy of a health intervention is assessed in an experiment-like design [20]. Subjects are randomly allocated to an intervention. Random allocation ensures that other exposures or potential causal factors of the outcome are equally distributed between the intervention and the control group. It avoids confounding and minimises bias. Therefore, RCTs are the reference standard to show causation in medical research. However, RCTs may not be feasible for every research question due to ethical issues [21].

Cross-sectional and longitudinal studies belong to the analytical epidemiological studies and are observational. In cross-sectional studies, a sample of the population at risk is observed at one point of time [22]. An example could be a survey that asks 1,000 people if they received a diagnosis of cancer or not to determine the prevalence rate of cancer in this population. In longitudinal studies, a group of people is prospectively followed over time [23]. Longitudinal studies allow calculating incidence rates, because new disease occurrences are registered.

More recent types of epidemiological studies include the case-crossover [24] and the case-time-control design [25,26]. In case-crossover studies, the case is used as its own control. This renders the case and control more similar. In case-time-control studies, the history of exposure of a conventional control group is used to adjust for the systematic error from temporal changes. These two study designs are often used in pharmacoepidemiological research [27,28].

1.1.3 Validity in epidemiological studies

Measurement errors define how well a study is capable of measuring what it intended to measure. A study is considered valid if it measured the truth or real situation in the population, no systematic errors are present and random errors are small [29].

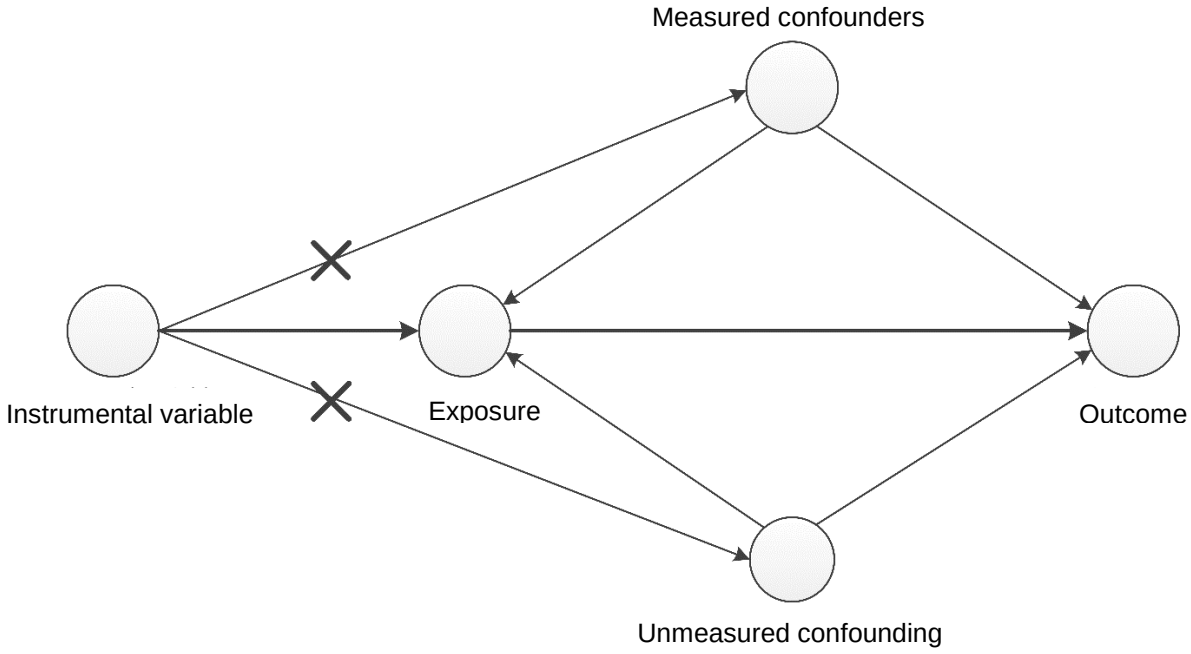
Random error can occur because in each study only a subsample of the entire population is included potentially leading to sampling error. Variation at random in individuals included in the study may also lead to random error. Random errors are less problematic for the validity of the study results than systematic errors.

Systematic error occurs if there is a systematic difference between what the study is estimating and what the study intended to estimate. It is more difficult to detect and to deal with systematic errors than random errors. There are two types of systematic errors, bias [30] and confounding [31].

Bias occurs if differences in the intervention and control group are introduced by uneven decisions or assessments made among the groups [32,33]. Examples of biases are detection bias (e.g. women see the physician more often than men), selection bias (e.g. patients with poor prognosis are selected as controls), attrition bias (e.g. patients among case group follow study protocol more closely than patients among control group), reporting bias (e.g. only selective outcomes are reported), loss to follow-up (e.g. study participants do not show up because they have moved) and measurement bias (e.g. physical assessment is more thoroughly performed in cases than controls) [30, 32, 33].

Confounding arises if other risk factors and potential causal factors of the outcome are not balanced between the case and control group [31]. A confounder is a factor that is associated with the exposure and with the outcome as presented in the directed acyclic graph (Figure 1.1-2) [34].

Figure 1.1-2: Directed acyclic graph (DAG)



In a study assessing the relationship between an exposure and an outcome, an instrumental variable is applied to remove confounding [35]. An instrumental variable is strongly associated with the exposure, but unrelated to confounders. Therefore, instrumental variables have only an effect on the outcome through their relationship with the exposure.

Confounding by indication refers to the fact that the clinical condition that determines the exposure is linked to the outcome, e.g. patients with more severe disease are more likely to receive the intervention and they have a higher risk of adverse outcome [36,37].

Interaction or effect modification describes how a relationship between an exposure and an outcome is changed by adding an effect modifier [36]. The effect modifier must be a risk factor for the outcome. For example, age or gender are effect modifiers for many conditions.

1.1.4 Basic epidemiological measures

The basis for epidemiological measures is the contingency table or also called 2x2 table [38] presented in Figure 1.1-3. This table can be extended if more categories are available.

Figure 1.1-3: Contingency table or 2x2 table

Outcome / **Exposure***	Cases	Controls	
Exposed	a (TP)	b (FP)	a+b
Non-exposed	c (FN)	d (TN)	c+d
	a+c	b+d	N=a+b+c+d

FN, false negative; FP, false positive; TN, true negative; TP, true positive

* or test result that can be positive or negative; or classification of the model that can be positive or negative

Prevalence proportions [39] are a measure of the outcome status and are defined as the proportion of subjects having the outcome in a given population at a single point in time ((a+c)/N).

Incidence proportions measure disease onset [39]. They can be calculated for the entire population ((a+c)/(a+b+c+d)) or separately for the exposed and the non-exposed groups by dividing the number of cases during a time period in the exposed and non-exposed group by the total number of exposed (a/(a+b)) or non-exposed subjects (c/(c+d)) during that time period in the study population, respectively. Relative risks (RRs) are derived by dividing the incidence proportions in exposed subjects by the incidence proportions in non-exposed subjects ((a/(a+b))/(c/(c+d))). Incidence rates (IRs) are calculated by dividing the number of cases by the total time contributed by the subjects followed. IRs can be calculated for the exposed and the non-exposed group and incidence rate ratios (IRRs) can be derived.

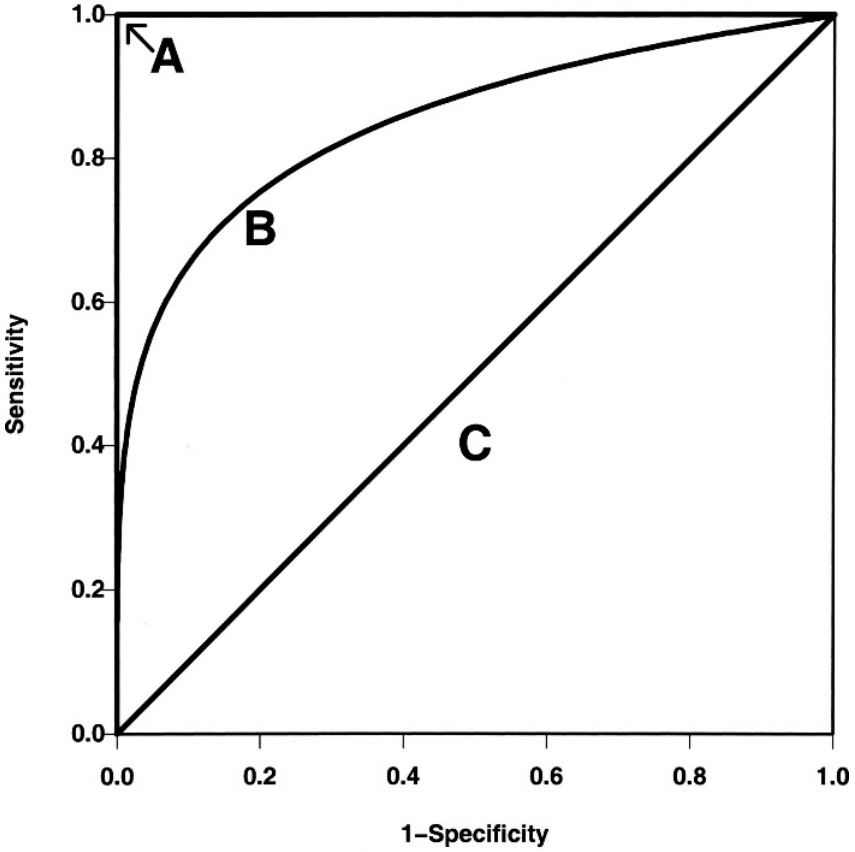
Because in case-control studies the number of cases and controls is arbitrary, no incidence proportions can be calculated. Instead, odds ratios (ORs) are calculated in case-control studies [40,41]. The odds of being exposed among cases or controls are calculated by dividing the number of exposed cases or controls by the number of non-exposed cases (a/c) or controls (b/d), respectively. ORs are derived by dividing the odds of being exposed among cases by the odds of being exposed among controls ((a/c)/(b/d))=ad/bc.

If the exposure variable is continuous, one option would be to categorise the variable and calculate ORs from the extended contingency table. Another option would be to compare means of the continuous exposure variable among the case and the control group. Continuous variables can also be included in regression models together with binary or categorical variables (see 3.2).

1.1.5 Diagnostic performance and predictive ability

In epidemiology, diagnostic performance of a test or predictive ability of a model can be evaluated by obtaining the following characteristics: sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) [42,43]. Sensitivity is the proportion of the persons with the outcome in the study population correctly identified as having the outcome (a/(a+c)). Specificity is defined as the proportion of subjects without the outcome in the study population correctly identified as not having the outcome (d/(d+b)). Sensitivity and specificity are not influenced by the prevalence of the outcome. The receiver operating characteristic (ROC) curve represents the diagnostic accuracy of a test or predictive ability of a model [44,45]. It is defined by sensitivity over 1-specificity (Figure 1.1-4). Or in other words, it is presented by the true positive rate over the false positive rate. The higher is the area under the ROC curve (AUC); the better is the test or model. If the AUC of the ROC curve has a value of 0.5, the discrimination of the test or model is no better than chance.

Figure 1.1-4: Receiver operating characteristic curve



Line A represents an area under the curve (AUC) of 1, which would indicate the highest diagnostic accuracy or predictive ability. The discriminating line C (AUC = 0.5) corresponds to a discriminative ability of random chance. B (AUC = 0.75) represents a good receiver operating characteristic curve. Source: Zou KH et al. Circulation. 2007;115:654-657

While sensitivity and specificity describe the overall characteristics of a test, the NPV and the PPV describe how a test or model works under specific circumstances or in a specific patient [43]. The NPV is the probability that subjects with a negative test result or classified as low risk by the model do not have the outcome (d/(c+d)) and the PPV is the probability that subjects with a positive test result or classified as high risk by the model have the outcome (a/(a+b)). These measures are influenced by the prevalence of the disease.

1.1.6 Analysis of epidemiological data

In an earlier section, bias [30] and confounding [31] were discussed. To avoid bias, a careful study design is important, because bias can usually not be addressed during data analysis. However, there are several options to control for confounding during data analysis such as stratification, restriction, matching, use of propensity score or multivariable regression analysis [46-50].

When the study population includes e.g. only male patients with the age between 50 and 60 years, restriction was applied [46]. This may limit generalisability of the results. Matching refers to the procedure whereby one or more controls are selected for each case on the basis of specific criteria such as age, gender, and other important potential confounders [47]. Potential confounders should then be equally distributed among groups. Rosenbaum

et al. defined the propensity score as the conditional probability of treatment or exposure given all confounders [48]. Even unmeasured confounders can be considered with propensity scores [49]. Stratification is an effective and straightforward way to control for confounding. It means that the data on exposure and outcome are presented by categories of one or more potential confounding variables and exposure-specific outcome measures can be presented [50]. Univariable analysis is a powerful method to initially screen the data or if only a few confounders need to be controlled for. Nevertheless, if we want to control for several confounders at the same time, multivariable regression analysis is the preferred option (see 3.2). Propensity scores can be included in multivariable regression analysis.

1.2 The burden of cancer

Diseases in which the control mechanisms during cell division are impaired are called cancers or malignant tumours [51]. Benign tumours do not spread to other parts of the body, whereas malignant tumours can invade every tissue of the body via the blood and lymph systems and cause metastases [51].

Cancers are usually named after the tissue they originate in. The following classification is suggested to divide main categories of cancer: carcinomas such as breast cancer, lung cancer and prostate cancer are cancers that derive from epithelial cells or tissues from internal organs; sarcomas are cancer cells that originate in connective or supportive tissue; myelomas are cancers of the bone marrow; lymphomas such as non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) originate in lymphatic tissue and leukaemias such as acute myelogenous leukaemia or chronic lymphocytic leukaemia (CLL) originate in tissue that produces blood cells [52]. However, in the future cancers may no longer be classified according to the tissue of origin, but according to the common present genetic mutation or tumour biomarker [53,54]. This approach is already applied for cancer treatment as genetic mutations and tumour biomarkers can determine the response to treatment [55].

About 30% of all cancer deaths are caused by preventable risk factors such as obesity, unhealthy diet, lack of physical activity, smoking and alcohol consumption [56]. In low- and middle-income countries, cancers that are caused by viral infections with hepatitis B and C virus or human papilloma virus are responsible for up to 20% of cancer deaths. About 60% of the new annual cancer cases occur in low- and middle-income countries of Africa, Asia, Central and South America [56].

In Europe, the age-adjusted incidence of common cancers such as colon, prostate, breast

and stomach cancer has slightly increased over the past two decades [57]. Although cancer mortality in high-income countries including Europe and the USA is declining [58,59], probably due to an earlier detection and better management of cancer, it still remains a leading cause of morbidity and mortality in Europe [58]. The Global Burden of Disease Study was initiated at the request of the World Bank and uses registration data and population-based study data sources to estimate worldwide and regional burden of disease in collaboration with the World Health Organization (WHO) [60]. It showed that global disability-adjusted life years in breast cancer and leukaemia were 174 per 100,000 population and 139 per 100,000 population in the year 2010, respectively [61]. Age-standardised mortality rates were 6.6 per 100,000 with breast cancer and 4.2 per 100,000 subjects with leukaemia [62].

1.2.1 Chronic lymphocytic leukaemia

CLL is a subtype of NHLs. It is the most common leukaemia in adults and constitutes approximately 1% of all cancers [63]. CLL is a blood cancer, which is characterised by the abnormal accumulation of B-cell lymphocytes in the bone marrow and blood [64]. B-cell lymphocytes belong to the white blood cells and play an important role for the immune system by recognising antigens and producing antibodies [65].

CLL is not a childhood disease and is very uncommon in young people. It becomes more common with older age, which is reflected by the average age at diagnosis of 72 years, and is more likely among men than women [66]. The estimated incidences for the USA and Europe range from 3-5 per 100,000 population or 100,000 person-years [66,67] and were determined before the year 2010.

CLL is clinically heterogeneous. The characterisation of CLL ranges from a slowly developing, asymptomatic lymphocytosis to a progressive disease with enlarged lymph nodes, splenomegaly, anaemia, and thrombocytopenia [68]. The majority of CLL patients, about 70%, have an asymptomatic, slowly progressing form with a long survival [63]. Because these patients feel well, they may get diagnosed with CLL after a visit to the general practitioner (GP), where blood samples have been taken for a routine check-up. Those patients usually do not require immediate treatment [64]. Others present with an aggressive, symptomatic leukaemia where immediate treatment is required to postpone further progression of the disease [64].

With standard chemotherapy CLL remains incurable. Treatment is palliative and should be delayed until diseased patients are symptomatic [69]. Combination chemotherapy with

fludarabine, cyclophosphamide and rituximab is considered as a standard for fit, chemotherapy naive patients [70]. Elderly or comorbid patients can be treated with chlorambucil or bendamustine [71]. In patients who do not respond to chemoimmunotherapy or have high-risk CLL, allogeneic hematopoietic stem cell transplantation has been considered a treatment option [72]. Those patients need to be healthy apart from CLL because the risks of a transplant in elderly and comorbid patients are not acceptable [73]. New emerging treatments such as the antibody obinutuzumab, the kinase inhibitor idelalisib, or the immunomodulator lenalidomide might be more tolerable treatment strategies in the future [74].

Several prognostic markers such as age, stage, performance status, lymphocyte count, serum parameters and chromosomal abnormalities have been identified [64]. For example, the expression of the ZAP-70 marker has been associated with a shorter time period until treatment is applied and a reduced overall survival (OS) [75]. Generally, survival of CLL patients ranges from less than 2 to more than 15 years and overall median survival is approximately 10 years [63]. Neutropenia and infections due to chemotherapy remain a major cause of morbidity and mortality in CLL [76].

1.2.2 Breast cancer

In the USA, one in eight women over their lifetime is affected by breast cancer which is the most common invasive cancer in women [77]. Breast cancer develops from breast tissue and can manifest as a lump in the breast, change in the shape of the breast, or other skin changes around the breast. It can also build metastases and grow into other tissues.

Incidence of breast cancer in Europe has been stable over the last 10 years or increased slightly due to implementation of breast cancer screening [57]. Other factors that contributed to the increase in breast cancer incidence are a change in lifestyle factors that are known to be associated with the risk of breast cancer and to a smaller extent genetic factors [78]. In Europe, the age-standardised incidence of breast cancer in 2008 was 70.7 per 100,000 women [79].

Causes of breast cancer are unknown. Several risk factors have been identified in breast cancer such as gender, age, family history, hormonal factors, genetic factors and lifestyle factors [80]. Male breast cancer accounts for approximately 1% of all breast cancers [81]. Most risk factors that have been identified for breast cancer in women are also applicable to breast cancer in men [82].

Treatment options for breast cancer are: treatment of local disease with surgery or radiation, and treatment of systemic disease with hormonal therapy, chemotherapy or immunochemotherapy, or a combination of these treatment options [83]. The need for treatment and selection of treatment option is based on patient age [84], hormone receptor status [85], tumour histology and pathology [86], human epidermal growth factor receptor 2 (HER2) status, and patient comorbid conditions [83].

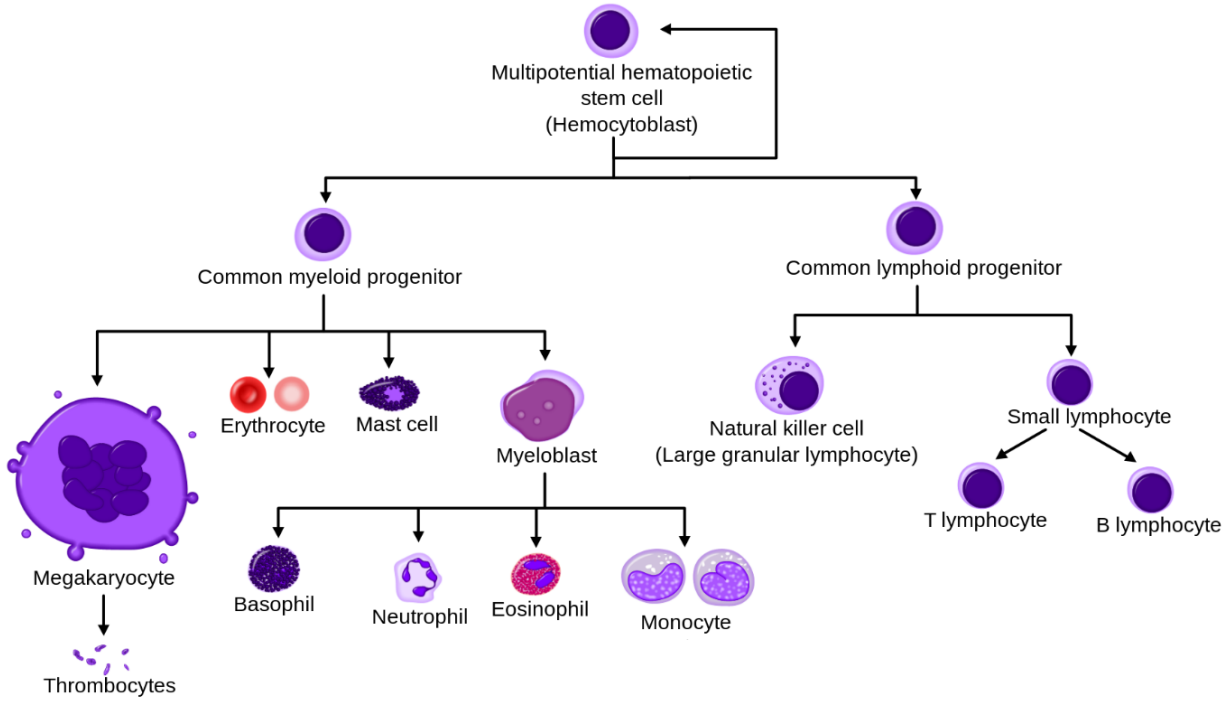
An indication for the prognosis of a patient with breast cancer is the TNM classification system; tumour size (T), nodes (N), and metastasis (M) [87]. The higher the tumour size and the more nodes involved, the less favourable is the prognosis. Patients with metastases have end-stage breast cancer. Two prognostic biomarkers such as estrogen receptor (ER) and HER2 are assessed routinely in every breast cancer to select patients benefitting from endocrine and HER2-targeted therapy [88]. Five-year disease-free survival rates differ according to breast cancer subtype and are about 93% in luminal A breast cancer and 78% in HER2-like breast cancer [89].

In breast cancer patients receiving chemotherapy, neutropenia is less common than in haematological cancer patients undergoing chemotherapy. But high neutropenia rates in breast cancer patients receiving a specific chemotherapy have been reported and can be reduced by using prophylactic granulocyte colony-stimulating factors (G-CSFs) [90].

1.3 Chemotherapy-induced and febrile neutropenia in cancer patients

Neutrophils belong to the white blood cells and are produced by haematopoietic stem cells (Figure 1.3-1). They form an important part of the innate immune system, because these are one of the first cells that enter the site of inflammation and release cytokines that amplify inflammatory reactions by other cell types [91].

Figure 1.3-1: Haematopoiesis



All blood cells develop and differentiate from pluripotent stem cells of the red bone marrow.

Source: Figure adapted from "Hematopoiesis simple" by Mikael Häggström (no attribution required), from original by A. Rad (requires attribution) - Image:Hematopoiesis_(human)_diagram.png by A. Rad. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons http://commons.wikimedia.org/wiki/File:Hematopoiesis_simple.svg#mediaviewer/File:Hematopoiesis_simple.svg

Neutropenia is defined as an absolute neutrophil count (ANC) lower than $1.5x10^9$/L and classified according to the severity of the reduction of the ANC [92]. An ANC less than $0.5x10^9$/L is defined as severe or grade 4 neutropenia, an ANC between $0.5x10^9$/L and $1.0x10^9$/L is defined as moderate neutropenia, and an ANC between $1.0x10^9$/L and $1.5x10^9$/L is defined as mild neutropenia. Chemotherapy-induced neutropenia (CIN) and febrile neutropenia (FN) are frequent complications in cancer patients undergoing chemotherapy [93]. FN is defined as an ANC lower than $0.5x10^9$/L with a concomitant oral temperature of 38° Celsius or more for more than 1 hour [94].

High rates of CIN, FN and infectious complications are observed in CLL, NHL and breast cancer patients receiving standard of care chemotherapy [90,95-98]. CIN and FN not only remain the principal dose-limiting toxicities for cancer chemotherapy [99], they may also affect short- and long-term outcomes. Patients experiencing neutropenic events are more susceptible to subsequent infections due to a low neutrophil count [99,100]. Chemotherapy dose reductions or delays and hospitalisations due to CIN or FN impact on treatment success and short-term mortality [101-103].

1.3.1 Risk factors

Risk factors of CIN or FN can be identified by performing univariable and multivariable regression analysis of collected data assessing the association between CIN or FN and different exposure variables.

Numerous risk factors in different types of cancer have been reported to increase the risk of FN. Those included patient-related, tumour-related and chemotherapy-related factors and were identified in univariable and multivariable analysis (controlling for confounders). Older age, lower weight, prior chemotherapy, higher planned dose of chemotherapy, higher number of planned chemotherapy cycles, higher chemotherapy intensity, vascular comorbidity, lower baseline with blood cell (WBC) and red blood cell (RBC) count, lower platelet and neutrophil count, and higher baseline bilirubin, low serum albumin or haemoglobin, anaemia, increased lactate dehydrogenase or alkaline phosphatase, abnormal liver or renal function, poor performance status, low lymphocyte count, tumour stage, and lack of G-CSF prophylaxis were shown to be associated with an increased risk of FN [104-120].

Chemotherapy dose delays and dose reductions before CIN or FN occurred, higher weight, and prophylaxis with antibiotics or G-CSFs have been reported to be protective factors [105,106,110,111,115].

More recently, genetic factors such as certain genotypes in *GSTP1* (Glutathione S-transferase P1)*, UGT1A1* (UDP glucuronosyltransferase 1A1)*, MBL2* (Mannose-binding lectin)*, ABCC1/MRP1* (multidrug resistance-associated protein)*, UGT2B7* (UDP glucuronosyltransferase 2B7) and *FGFR4* (fibroblast growth factor receptor 4) were shown to be significant predictors of FN in various tumour types [110,121-126].

1.3.2 Prediction models

The underlying analysis to determine risk factors or to develop risk prediction models is the same. For the development of a risk prediction model, as many identified risk factors as possible should be considered.

Risk models for the occurrence of CIN [106] and FN in breast cancer [104,127] and NHL patients [105] including patient- or chemotherapy-related factors have been published and were reported to be predictive. Other neutropenia risk models in different cancers have been proposed [113,128-131]. Risk factors that were reported in most studies were low

WBC count, planned cycles of chemotherapy, and higher intensity of chemotherapy. Several risk prediction models assessed model performance by reporting test characteristics and ROC curves [105,106,113,127,130]. Ranges were 24% - 90% for sensitivity, 59% - 93% for specificity, 84% - 97% for NPV, 12% - 59% for PPV, and 0.74 - 0.86 for area under the ROC curve [105,106,113,127,130,131]. All models had in common that they were predictive of the outcome to a certain extent, but the PPV remained low. Further refinement of these models is necessary.

Different approaches for internal validation such as split-sample validation, cross-validation, and bootstrapping are available [132]. Hosmer et al. and Lyman et al. [113,130] split their population sample into a training dataset, where the model was developed and a validation dataset, where the developed model was applied to. Pettengell et al. and Schwenkglenks et al. used 10-fold cross validation [105,106]. The advantage of 10-fold cross validation is that the entire sample is used to either develop or validate the model. The dataset is randomly split into 10 subsamples and the model is developed in 9 datasets and validated in the remaining dataset [133]. This procedure will be repeated for each subsample [133]. Dranitsaris et al. used bootstrapping techniques to show that the resampled bootstrap regression coefficients and confidence intervals (CIs) were similar to the regression coefficients and CIs obtained from the model [127,132]. Predictive ability of all models during split-sample validation and cross-validation was slightly lower than in the apparent dataset.

Before risk prediction models can be applied in clinical practice they should undergo external validation [134]. Jenkins et al. performed a partial validation of their original model [104] using an independent dataset [108]. In addition, the Jenkins' model [104] was externally validated by other researchers, who concluded that the Jenkins' model cannot accurately identify patients at high risk of FN, but no successful validation criteria were pre-defined [135].

1.4 Granulocyte colony-stimulating factors

Natural human G-CSF is a bone marrow-stimulating hormonal glycoprotein that induces the proliferation and differentiation of pre-mature granulocytes and other haematopoietic stem cells [136]. Granulocyte macrophage colony-stimulating factors have haematopoietic activity, but are reported to be less efficient than G-CSFs [137]. Natural G-CSFs are produced by several cells of the immune system [136].

Pharmaceutical analogues of naturally occurring G-CSFs called recombinant human G-CSFs such as filgrastim and pegfilgrastim have been developed [138,139]. They have been shown to stimulate the production and differentiation of neutrophils. Two main groups of G-CSFs can be differentiated: short-acting G-CSFs such as filgrastim, lenograstim, and sargramostim and long-acting G-CSFs such as pegfilgrastim, lipegfilgrastim and balugrastim [140]. Daily G-CSFs are primarily cleared through the kidneys and require daily dosing until recovery of the neutrophil count. Long-acting G-CSFs are primarily cleared by neutrophils and have significantly reduced renal clearance compared with daily G-CSFs. They therefore require only a single dose per chemotherapy cycle.

G-CSFs have been shown to reduce the incidence and duration of CIN and FN [140]. According to the European Organisation for Research and Treatment of Cancer (EORTC) and other international guidelines, prophylactic G-CSF use is recommended if the underlying risk of FN of the planned chemotherapy regimen is 20% or higher [98,141-143]. For chemotherapy regimens with an intermediate FN risk (10-20%), the EORTC guideline recommends that patient risk factors should be taken into account when the individual risk of FN and the likely benefit of G-CSF support is determined [98]. For patients with prolonged neutropenia or other risk factors favouring neutropenia-related complications, antibacterial and antifungal prophylaxis have recently been recommended [144]. But issues with resistance need to be considered.

2 Objectives of the thesis

The overall aim of this thesis was to contribute to the knowledge of CLL epidemiology and CIN or FN occurrence in cancer patients, and to promote the development and validation of risk prediction models for FN to optimally target G-CSF prophylaxis in cancer patients undergoing chemotherapy. Following research questions were defined for the four subsequent studies.

The aim of the first study was to assess incidence rates of CLL between the years 2000 and 2012 and to evaluate time trends in CLL incidence. Another aim of the study was to describe medical resource utilisation in CLL patients and to derive changes over time.

In the second study, the author aimed to describe the occurrence of FN in breast cancer patients in the first and any cycle of chemotherapy. Based on a large set of patient-related, chemotherapy-related, tumour-related factors as well as genetic characteristics, a risk prediction model for the occurrence of FN was developed.

The research question of the third study was to externally validate the predictive ability of a risk prediction model for FN in NHL patients developed by the Impact of Neutropenia in Chemotherapy-European Study Group (INC-EU) using an independent NHL dataset.

By conducting a systematic literature review, the fourth study summarised the available evidence on the efficacy, effectiveness and safety of long-acting G-CSFs for prophylaxis of CIN and FN in adult cancer patients undergoing chemotherapy.

3 Methods

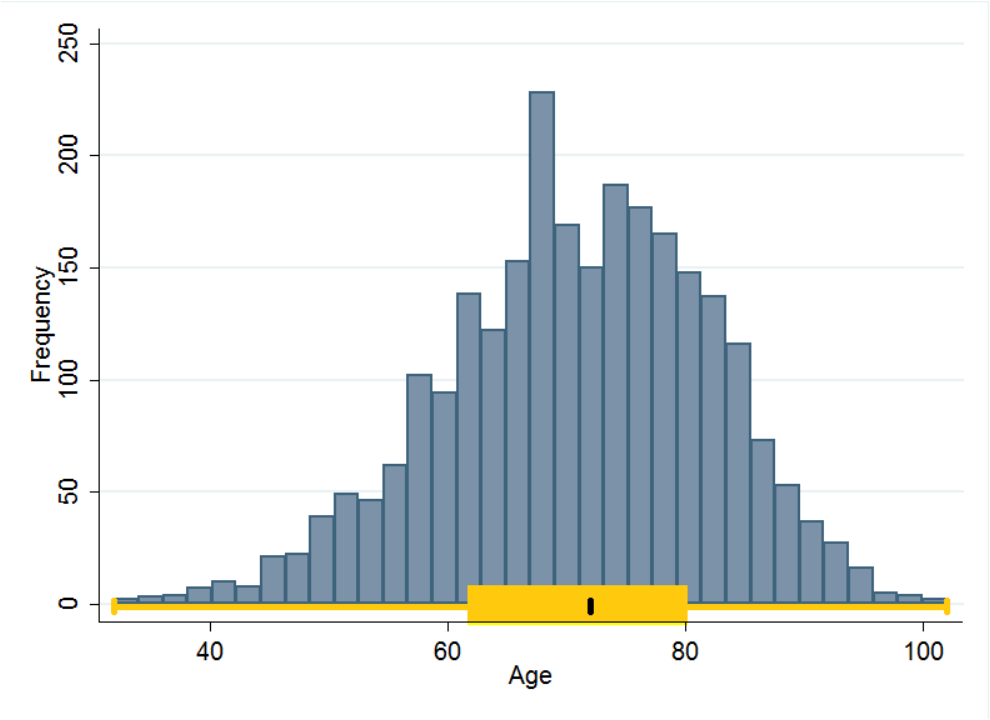
The studies which form the basis of this work were conducted using different data sources. For the first study, a physician-based large healthcare database was used, which contains electronically recorded patient data about demographics, diagnoses, healthcare visits and prescriptions (4.1). The second analysis was conducted using a hospital-based database, which followed a cohort of a pre-defined population over a certain time period and collected data about several exposures (4.2). For the third analysis, collected data from two independent prospective observational studies looking at a specific outcome were evaluated (4.3). In the last study, electronic databases of published literature were used to search for studies on specific drugs (4.4). Details of the data sources are described in the sections 4.1 - 4.4 and are provided for each study separately.

Different methodological approaches were used. Qualitative methods for data collection and data analysis such as systematic literature reviews were combined with descriptive and quantitative methods such as univariable and multivariable regression analysis. The general principles of the methods applied are described in the following sections. Details of the methodological approaches are reported in the results' sections 4.1 - 4.4 and according to the studies.

3.1 Descriptive and univariable analyses

The aim of descriptive analysis was to provide an overview of the data and population studied. A common way is to provide tables that contain quantitative information about the most important exposure and/or outcome variables. Basic descriptive statistics that were used in the studies included the number of observations (N for total number of e.g. subjects or participants included, n for the number of observations); mean and standard deviation (SD) for normally distributed numerical and continuous variables; median, quartiles and range for numerical and continuous variables with a skewed distribution; and frequencies and percentages for binary (e.g. yes or no), categorical (e.g. blue, green, or red) or ordered variables (e.g. mild, moderate, severe). These data can be graphically represented by e.g. histograms or boxplots (Figure 3.1-1).

Figure 3.1-1: Histogram and corresponding boxplot



A histogram (dark grey) represents the probability distribution of a continuous variable. A boxplot indicates the median (black) and the spread of the data (light grey).

Univariable analysis is appropriate to measure the association between the outcome and one exposure variable at the time. For binary or categorical data, a chi-squared test was performed. The chi-squared test assessed if there is a difference in two or more proportions [145]. If sample sizes would have been small, Fisher's exact test would have been used instead. Linear correlations between exposure variables were assessed using Pearson's correlation coefficient and monotonic correlations were assessed using Spearman's correlation coefficient. Univariable logistic regression was performed to evaluate associations between the outcome and continuous variables and effect measures (e.g. ORs and 95% CIs) and p-values were obtained.

3.2 Multivariable regression analysis

Multivariable regression analysis is a powerful technique to describe the association between the outcome variable and several exposure variables or to predict the outcome based on the exposure variables. Depending on the aim of the regression model, different model development approaches are recommended. To determine factors that could predict the outcome, as many variables as necessary to get a reliable prediction model should be included. For a potentially causal model, only those factors with a specific hypothesis for a relationship with the outcome should be included. Drawing a DAG beforehand can help to identify those factors. The choice of regression analysis depended on the type of outcome variable that was assessed and its underlying distribution. For

example, linear regression analysis is used when the outcome variable is continuous [146].

$$Y\ (outcome) = \alpha\ (intercept) + \beta(regression\ coefficient) * X\ (exposure\ variable)$$

Other regression analyses such as logistic regression, Poisson regression, and Cox proportional hazard regression applied in the studies this work is based on are described in the following sections.

3.2.1 Multivariable logistic regression

The use of logistic regression analysis is appropriate when the outcome variable is binary (e.g. yes or no) [147]. In logistic regression, the proportion of the outcome variable Y is assessed given the exposure variable X. Because proportions range from 0 to 1, but the predicted values in standard multiple regression can take any negative or positive value, it is necessary to transform the outcome variable. The proportion of the outcome is transformed into odds (proportion [p]/1-proportion [1-p]) and we take the log of the odds which is called logit transformation.

$$\log(\frac{p}{1-p}) = \alpha\ (intercept) + \beta(regression\ coefficient) * X\ (exposure\ variable)$$

For the logistic model, the maximum likelihood method provides estimates of the regression coefficients (α, β) which maximise the likelihood of obtaining the data that were observed.

As the logistic regression model was fitted using the logit transformation, the regression coefficients need to be exponentiated before being interpreted. When we exponentiate the regression coefficient β, we obtain the OR of occurrence of the outcome for a one unit increase of the exposure variable (continuous) or the OR for a certain group compared to the reference group (binary or categorical variable).

To test whether the logistic regression model including the exposure variable fits the data better than the model without the exposure variable, a likelihood ratio test can be obtained. If the likelihood ratio test reports a p-value<0.05, it means that the exposure variable would usually be included in the model.

The same rules apply to multivariable logistic regression. Instead of only one exposure variable, several exposure and confounding variables can be included in the model.

$$\log(\frac{p}{1-p}) = \alpha\ + \beta 1 * X1 + \beta 2 * X2 + \cdots + \beta n * Xn$$

The interpretation of the OR is the same, except that the resulting OR is adjusted for other exposure variables and confounding variables.

3.2.2 Poisson regression

Poisson regression is used if the outcome variable describes counts or rates, which are distributed at random and independent of each other [148]. The analysis of counts with Poisson regression shares common features with the logistic regression. As with the logistic regression, the right part of the equation can take more values than the left part of the equation. Therefore, the left part of the model is log-transformed. This kind of Poisson regression model is called log-linear model.

$\log(Y) = \alpha + \beta * X$ which is equivalent to $Y = \exp(\alpha + \beta * X)$

The regression coefficients of the Poisson regression are calculated using again the maximum likelihood method. Because of the log transformation, the resulting regression coefficients need to be exponentiated for interpretation. The exponentiated regression coefficient α estimates the mean of the outcome variable when the exposure variable is zero. With every one unit increase of the exposure variable, the outcome is multiplied by the exponentiated regression coefficient β. To test if the exposure variable should be included in the model or not, the likelihood ratio test can be applied.

Rates such as incidence rates or mortality rates are analysed using Poisson regression as well. In addition to counts, rates also take into account follow-up time or the period of exposure and are defined as number of counts (N) divided by the time period (T). Using again the log transformation, the Poisson regression takes the following form:

$\log(R) = \log\left(\frac{N}{T}\right) = \alpha + \beta * X$ which is equivalent to $\log(N) = \log(T) + \alpha + \beta * X$

The offset is the log of the time period, which is considered as a variable in the Poisson regression with a fixed regression coefficient 1. By exponentiating the regression coefficient it can be interpreted as rate ratio or IRR for a one unit increase of the exposure variable or the rate ratio or IRR for a certain category compared to the reference category.

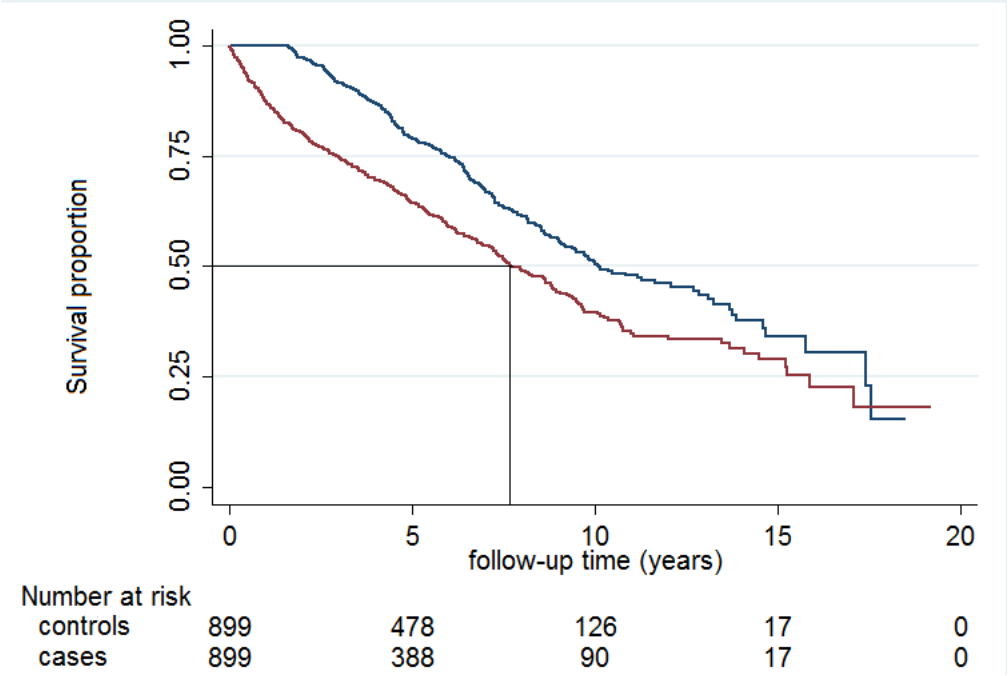
3.2.3 Cox proportional hazard regression

Cox proportional hazard regression is applied when analysing time-to-event or survival data [149]. Survival is a function of time: it is 1 at the time point 0 and it goes to 0 as time goes to infinity. The hazard function h(t) is the instantaneous rate at which events occur

given no previous events. The cumulative hazard H(t) describes the accumulated hazard up to time t.

Survival can be estimated by using the non-parametric Kaplan Meier estimator (Figure 3.2-1). At every time point, the Kaplan Meier estimator calculates the number of subjects still at risk, the number of subjects that had the outcome, and the number of subjects that have been censored. From these numbers, the Kaplan Meier approach can estimate the probability of survival and the survival function. But the Kaplan Meier curve is not suitable for the evaluation of the impact of several exposures on survival.

Figure 3.2-1: Kaplan-Meier estimate of survival



Kaplan Meier curve presenting survival curves for a non-exposed group (line above) and an exposed group (line below). Median survival is 7.6 years for the exposed group and 10 years for the non-exposed group.

The most commonly used regression for survival data is the Cox proportional hazards regression. The Cox proportional hazard model assumes that without interaction, the exposures and confounders add to the baseline hazard and the effect of the exposures does not change over time. The regression takes the form:

$h(t|X) = h(t)$(baseline hazard) $* \exp(\alpha + \beta * X)$ (effect of exposures)
which is equivalent to $\log\big(h(t|X)\big) = \log\big(h(t)\big) + (\alpha + \beta * X)$

By exponentiating the regression coefficient it can be interpreted as hazard ratio (HR). For every one unit increase of the exposure variable, the hazard of the event increases by the factor of the HR at all points in time.

3.3 Systematic literature review

A systematic literature review aims to summarise the available evidence regarding a specific topic of interest. It aims to provide an objective appraisal of the evidence. In comparison to narrative literature reviews, it follows a systematic approach to minimise bias and random errors. This means that a systematic review should always include a materials and methods' section. Guidelines on the conduct of systematic reviews have been published (Table 3.3-1) [150,151].

Before conducting a systematic literature review, a study question needs to be formulated according to the PICOS (P-who is the patient/participant, I-what is the intervention or exposure, C-what is the comparison group, O-what is the outcome or endpoint, S-what is the study design) criteria, which are an expansion of the PICO criteria [152].

After the study question has been defined, a search strategy needs to be developed considering as much literature sources as appropriate including e.g. MEDLINE, EMBASE, the Cochrane Library, the Centre for Review and Dissemination databases, the Cochrane Central Register of Controlled Trials, other trial registers, manual searches of key journals and abstract books. Keywords or search terms need to be defined that are used to search the databases. Eligibility criteria have to be defined beforehand to decide which of the studies will be included in the systematic literature review. Study selection and data extraction should be performed by two independent researchers, if possible. For the analysis and interpretation of the results, a risk of bias assessment of the included studies is recommended to consider limitations, biases, strength of the evidence and applicability. Finally, the results of the systematic literature review should be published.

Although the primary analysis of systematic literature reviews is of qualitative nature, there is a method called meta-analysis that quantitatively summarises the data obtained from a systematic literature review. However, if the included studies are very heterogeneous, guidelines do not recommend performing meta-analysis [153].

Table 3.3-1: PRISMA 2009 Checklist [151]

Section/topic	#	Checklist item	Reported on page #
TITLE	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS) and any assumptions that were made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding and role of funders for the systematic review and other support	

4 Published results

According to the four studies that formed the basis for this thesis, the results' section is divided into four parts (4.1 - 4.4). Each part corresponds to one peer-reviewed publication.

4.1 Trends in incidence and medical resource utilisation in patients with chronic lymphocytic leukaemia: insights from the UK Clinical Practice Research Datalink (CPRD). Ann Hematol. 2014 [Epub ahead of print]

4.2 Multivariable regression analysis of febrile neutropenia occurrence in early breast cancer patients receiving chemotherapy assessing patient-related, chemotherapy-related and genetic risk factors. BMC Cancer 2014; 14: 201

4.3 External validation of a risk model of febrile neutropenia occurrence in patients with non-Hodgkin lymphoma. Leuk Lymphoma 2013; 54(11): 2426-2432

4.4 Efficacy, effectiveness and safety of long-acting granulocyte colony-stimulating factors for prophylaxis of chemotherapy-induced neutropenia in patients with cancer: a systematic review. Support Care Cancer 2015; 23(2): 525-545

For all publications, final drafts after refereeing are included below as permitted by the publishers' copyright and self-archiving policies. Tables and figures have been numbered according to the section to maintain the numbering within a publication. Reference lists are provided for each peer-reviewed publication separately and a comprehensive list of references is provided at the end.

4.1 Trends in incidence and medical resource utilisation in patients with chronic lymphocytic leukaemia: insights from the UK Clinical Practice Research Datalink (CPRD)

Alena M. Pfeil[1], Patrick Imfeld[2,3], Ruth Pettengell[4], Susan S. Jick[5], Thomas D. Szucs[1], Matthias Schwenkglenks[1]

1 Institute of Pharmaceutical Medicine (ECPM), University of Basel, Basel, Switzerland
2 Basel Pharmacoepidemiology Unit, Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland
3 Hospital Pharmacy, University Hospital Basel, Basel, Switzerland
4 Cellular and Molecular Medicine, St. George's University of London, London, UK
5 Boston Collaborative Drug Surveillance Program, Boston University School of Public Health, Lexington, MA, USA

Corresponding author:
Prof. Dr. Christoph R. Meier, Basel Pharmacoepidemiology Unit & Hospital Pharmacy, University Hospital Basel, Spitalstrasse 26, CH-4031 Basel, Switzerland, Phone: +41 61 556 53 69, Fax: +41 61 265 88 75, Email: christoph.meier@usb.ch

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Abstract

Background: Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in European adults. We aimed to evaluate time trends in CLL incidence and medical resource utilisation of CLL patients in the UK.

Methods: We conducted a retrospective, observational cohort analysis using the UK Clinical Practice Research Datalink (CPRD) comprising mainly primary care data. We included adult patients with newly diagnosed CLL between January 2000 and June 2012. We performed descriptive and trend analyses of CLL incidence and medical resource utilisation.

Results: A total of 2,576 patients with CLL met eligibility criteria. At diagnosis, the majority of patients (71.7%) were above 65 years of age. The European age-standardised CLL incidence rate in the CPRD was 6.2/100,000 (95% confidence interval [CI] 6.0, 6.5/100,000) person-years. There was no statistically significant increase over time. The CLL patients had on average 74.6 general practitioner visits during a median follow-up of 3.3 years. Between 2000 to 2012, the average number of recorded hospitalisations and referrals per year corrected for duration of follow-up significantly ($p<0.001$) increased by 8.1% (95% CI 6.8%, 9.3%) and 16.4% (95% CI 15.4%, 17.3%), respectively. Referrals and hospitalisations in the second year compared to the first year following the CLL diagnosis significantly decreased.

Conclusion: CLL incidence rates in the CPRD were stable over the period from 2000 to 2012. Medical resource utilisation in UK primary care was well documented, but further research is needed to describe secondary and tertiary care medical resource utilisation, e.g. chemotherapy.

Introduction

Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in adults. The clinical manifestations of CLL range from an asymptomatic presentation with minimal B-cell lymphocytosis to a progressive clinical picture of enlarged lymph nodes, splenomegaly, anaemia, and thrombocytopenia [68]. Median age at diagnosis has been reported to be 72 years [66] and males are more likely to develop CLL than females. Several prognostic markers such as age, stage, performance status, lymphocyte count, serum parameters and chromosomal abnormalities have been identified [68]. Survival time of patients with CLL ranges from less than two years to 15 years or more with a median survival of about 10 years [63]. In the US, CLL occurs with an estimated incidence of 3-5/100,000 people per year [66]. A crude incidence rate of 4.2/100,000 person-years has been reported for the UK in 2009 [67]. There are no current age-standardised incidence data for Europe.

About 70% of patients with CLL are asymptomatic and at an early disease stage at the time of diagnosis [63]. Chemotherapy in CLL patients is not recommended until patients develop symptoms, have organ compromise or until the disease is rapidly progressing. Before initiating chemotherapy treatment, it is important to assess the fitness level of a CLL patient [154,155]. Depending on life expectancy, general health status and expected ability to tolerate aggressive chemotherapy, different chemotherapy regimens are used to treat CLL. Combination chemotherapy with fludarabine, cyclophosphamide and rituximab is considered as a standard for fit, chemotherapy naive patients [156] but may not always be suitable for elderly patients or those with comorbidities. Chlorambucil in combination with an anti-CD20 monoclonal antibody such as rituximab or obinutuzumab is considered standard for elderly or comorbid patients [157].

As a result, various management strategies are possible. Intensive immuno-chemotherapy is given to fit patients with no or mild comorbidities and normal life expectancy (Go-Go) aiming at achieving prolonged progression-free and overall survival. Symptom relief and response to treatment are the main goal in the intermediate chemotherapy intensity group with moderate comorbidities and compromised life expectancy (Slow-Go). In patients with several or severe comorbidities and very short life expectancy, symptom management and prolongation of functional independence (No-Go) are the main objectives [158]. Instead of starting with the least aggressive chemotherapy, followed if needed by a second, more aggressive treatment regimen, the optimal chemotherapy is given to patients upfront to

keep them in remission for as long as possible. The change in management of CLL patients from palliative to curative care and towards an individually-tailored chemotherapy [64,159], together with its impact on resource utilisation, are poorly studied.

We aimed to assess CLL incidence rates and to evaluate time trends in CLL incidence and medical resource utilisation of CLL patients using a primary care database from the UK.

Methods

Study design and data source

We conducted a retrospective, observational cohort study using the large, anonymised UK-based Clinical Practice Research Datalink (CPRD). The CPRD, formerly known as the General Practice Research Database (GPRD), established in 1987, is part of the UK National Health Service's (NHS) National Institute for Health Research (NIHR) and the Medicines and Healthcare Products Regulatory Agency (MHRA). The database contains primary care data on about 13% of the UK population and is representative of this population with respect to age, gender, regional distribution and annual turnover rate. General practicioners (GPs) are trained to record clinical data in a standardised manner. The MHRA anonymises the raw data before release and performs quality controls to ensure that the standards of the data collection are fulfilled. The database contains information on patient demographics such as age and gender, body mass index (BMI), region of residence, medical diagnoses, laboratory test results, information about referrals to specialists, hospitalisations, and drug prescriptions.

This study was approved by the Independent Scientific Advisory Committee (ISAC) for MHRA database. Numerous studies involving cancer have been conducted using the CPRD [160-162], and the validity of the data recorded in the database has been reported to be high [163].

Study population

The study population consisted of patients with a diagnosis of CLL between January 2000 and June 2012 aged 18 years or older at the time of diagnosis. Subjects with less than three years of recorded history prior to the CLL diagnosis were excluded in order to increase the likelihood of capturing truly incident cases. Patients diagnosed with CLL before 2000 were excluded because the degree of consistency of the recording of the CLL diagnosis and the medical resource utilisation in the CPRD before 2000 was unclear. We

validated the CLL diagnosis by screening 100 randomly selected CLL patient records for typical diagnostic procedures such as blood tests, chest X-rays, bone marrow examinations and ultrasound scans; for symptoms such as swollen lymph glands, fever, unusual sweating and tiredness; for referrals to a specialist or specialised clinic; and for prescriptions such as chlorambucil, fludarabine, cyclophosphamide and rituximab. The CLL diagnosis was considered validated if the 100 CLL patient profiles included at least one screening criterion for CLL.

Follow-up and incidence rates

We followed all patients in the study population from the date of CLL diagnosis until they died, left the practice, or reached the end of the study period. Incidence rates were calculated as the number of new cases divided by the total number of person-years at risk. We summarised person-years by year for all subjects enrolled in the CPRD with at least three years of prior history and at risk of CLL between 2000 and the end of follow-up (i.e. a CLL diagnosis, death, leaving the practice, or the end of the study period). Age-standardised incidence rates were calculated based on the most recently updated European standard population [164].

Basic characteristics and Charlson comorbidity weighted index

For all patients in the study population, we assessed basic characteristics such as age at CLL diagnosis, gender, smoking status, alcohol consumption, BMI, body surface area (BSA), height and weight. For smoking status, alcohol consumption, BMI, BSA, height and weight the values recorded closest and prior to the CLL diagnosis date were extracted. Additionally, we extracted all comorbidities included in the Charlson comorbidity weighted index, because they were associated with the risk of mortality [165].

Medical resource utilisation

We assessed medical resource utilisation between the CLL diagnosis and the end of follow-up, including number of GP visits, hospitalisations, referrals to specialists (such as oncologists, radiotherapists, or haematologists) or specialised clinics, number of cancer-related therapies (such as chemotherapies or radiotherapies), blood transfusions, blood tests (such as red blood cell counts or neutrophil counts), and prescriptions for chemotherapeutics, antidepressants, antiemetics, antifungals, antibiotics, antivirals, immunosuppressants and growth factors.

Descriptive and statistical analysis

We summarised categorical data using standard descriptive statistics, i.e. number of observations, frequencies and percentages. Continuous data were reported using the mean and standard deviation (SD) if normally distributed. Median and interquartile range (IQR) or range were reported if the distribution was skewed. We performed log-linear poisson regression to describe the change in age-adjusted incidence rate over time [166]. The age-specific inidence rates, expressed as the division of incident counts and corresponding person-years, were log-transformed. Person-years on the log scale were then added up and used in the model as an offset, a value that is known and not predicted by the regression. The offset was subsequently used to re-calculate the age-adjusted incidence rate from the estimated count.

We performed multivariable Cox regression [149] to determine the influence of age at diagnosis, gender, Charlson comorbidity weighted index and year of diagnosis on overall survival. Analyses of time trends in medical resource utilisation were performed using poisson regression for count data. To correct for a possible cohort effect in the CPRD trend analyses of medical resource utilisation, years of follow-up were included in the Poisson regression model as an explanatory variable.

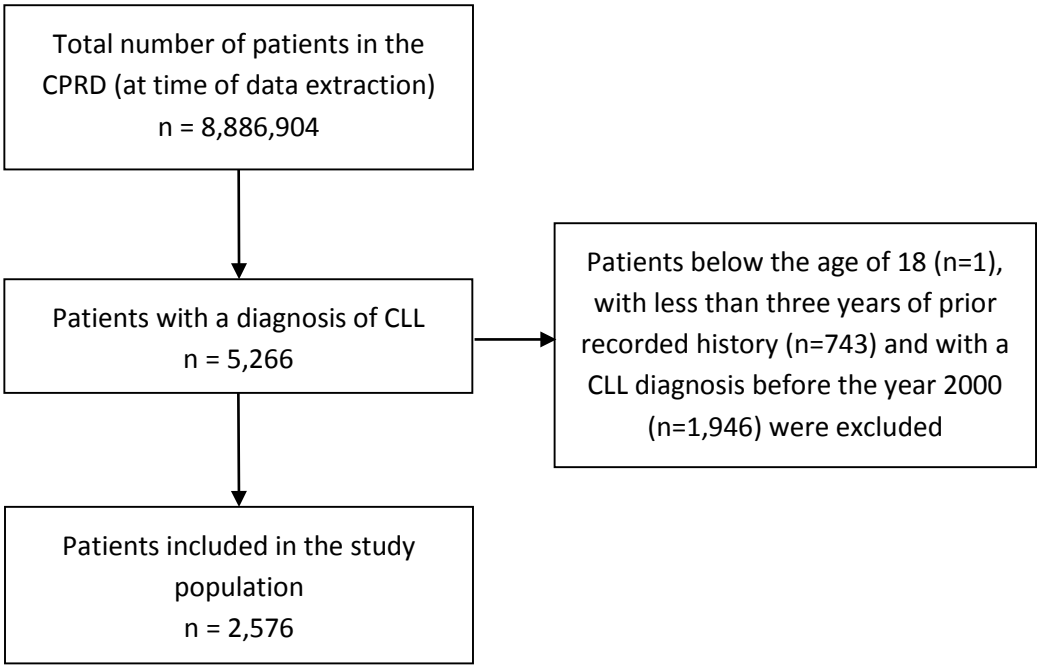
We carried out two-sided statistical tests at a 5% significance level and 95% confidence intervals (CIs) were obtained, if applicable. All analyses were performed using Stata/SE version 12.1 (StataCorp LP, College Station, TX, USA).

Results

We identified 5,266 (0.05%) patients with a diagnosis of CLL from among around 9 million subjects currently included in the CPRD. Of these, 2,690 patients were excluded because they did not meet eligibility criteria. Figure 4.1-1 shows details of the patient selection.

We included 2,576 patients with a diagnosis of CLL aged 18 years or older in the analyses, of whom 1,498 (58.2%) were male and 1,078 (41.8%) were female. Mean age at diagnosis was 70.9 years (SD±11.3 years, range: 32-102 years); a majority of patients (n=1,847, 71.7%) were of age 65 years or over. On average, CLL patients had 12.8 (SD 4.9) years of recorded prior history. Further characteristics of the study population are presented in Table 4.1-1.

Figure 4.1-1: Patient selection flow diagram



Incidence rates

The overall crude CLL incidence rate was 7.2/100,000 (95% CI 6.9, 7.4/100,000) person-years, and the overall age-standardised incidence rate was 6.2/100,000 (95% CI 6.0, 6.5/100,000) person-years. Age-standardised incidence rates in 2000 and 2010 were 5.6/100,000 (95% CI 4.7, 6.4/100,000) person-years and 6.4/100,000 (95% CI 5.5, 7.2/100,000) person-years, respectively (Figure 4.1-2). This corresponds to an average annual increase of 0.2%, which did not reach statistical significance (p=0.787). In some years, rates of up to 6.8/100,000 (95% CI 5.9, 7.7/100,000) person-years were seen. There was a decline in the age-standardised incidence rate in 2012. In the past five years, we observed a stabilisation of CLL incidence rates in the CPRD population.

Table 4.1-1: Characteristics of the study population

		Patients	
		N = 2,576	**%**
Age at diagnosis		2,576	100.0
18-32 years		0	0.0
32-62 years		607	23.6
63-71 years		672	26.1
72-78 years		598	23.2
79+ years		699	27.1
Gender		2,576	100.0
Female		1,078	41.8
Male		1,498	58.2
Smoking status		2,576	100.0
Non-smoker		1,156	44.9
Current smoker		252	9.8
Former smoker		1,053	44.0
Unknown		33	1.3
Alcohol consumption		2,576	100.0
Never		439	17.0
Current		1,846	71.7
Former		65	2.5
Unknown		226	8.8
Body mass index (kg/m^2) 26.6 ± 4.8*		2,388	92.7
Body surface area (m^2) 1.9 ± 0.2*		2,384	92.5
	n	**Men (%)**	**Women (%)**
Comorbidities			
None	1,529	873 (33.9)	656 (25.5)
COPD	189	136 (5.3)	53 (2.0)
Congestive heart failure	95	64 (2.5)	31 (1.2)
Myocardial infarction	65	46 (1.8)	19 (0.7)
Hypertension	406	221 (8.6)	185 (7.2)
Hypotension	55	37 (1.4)	18 (0.7)
Diabetes	215	127 (4.9)	88 (3.4)
Peripheral vascular disease	82	53 (2.1)	29 (1.1)
Cerebrovascular disease	73	44 (1.7)	29 (1.1)
Renal diseases	392	209 (8.1)	183 (7.1)
Ulcer	187	105 (4.1)	82 (3.2)
Liver disease	8	8 (0.3)	0 (0.0)
Connective tissue disease	3	3 (0.1)	0 (0.0)
Hemiplegia	2	2 (0.0)	0 (0.0)
Dementia	60	28 (1.1)	32 (1.2)
All tumours	7	5 (0.2)	2 (0.0)
Metastatic solid tumour	7	5 (0.2)	2 (0.0)
Lymphoma#	86	53 (2.1)	33 (1.3)
Secondary malignancies§	127	84 (3.3)	43 (1.7)

	n	Men (%)	Women (%)
Charlson comorbidity weighted index			
0	1,529	873 (33.9)	656 (25.5)
1	424	255 (9.9)	169 (6.6)
2	376	220 (8.5)	156 (6.1)
3	151	91 (3.5)	60 (2.3)
4	52	31 (1.2)	21 (0.8)
5	18	12 (0.5)	6 (0.2)
6	15	8 (0.3)	7 (0.3)
7	4	3 (0.1)	1 (0.0)
8	5	4 (0.2)	1 (0.0)
9	2	1 (0.0)	1 (0.0)

COPD, chronic obstructive pulmonary disease; n, number of patients; NOS, not otherwise specified

* mean ± standard deviation

% given as the percentage of the total number of patients (n=2,576)

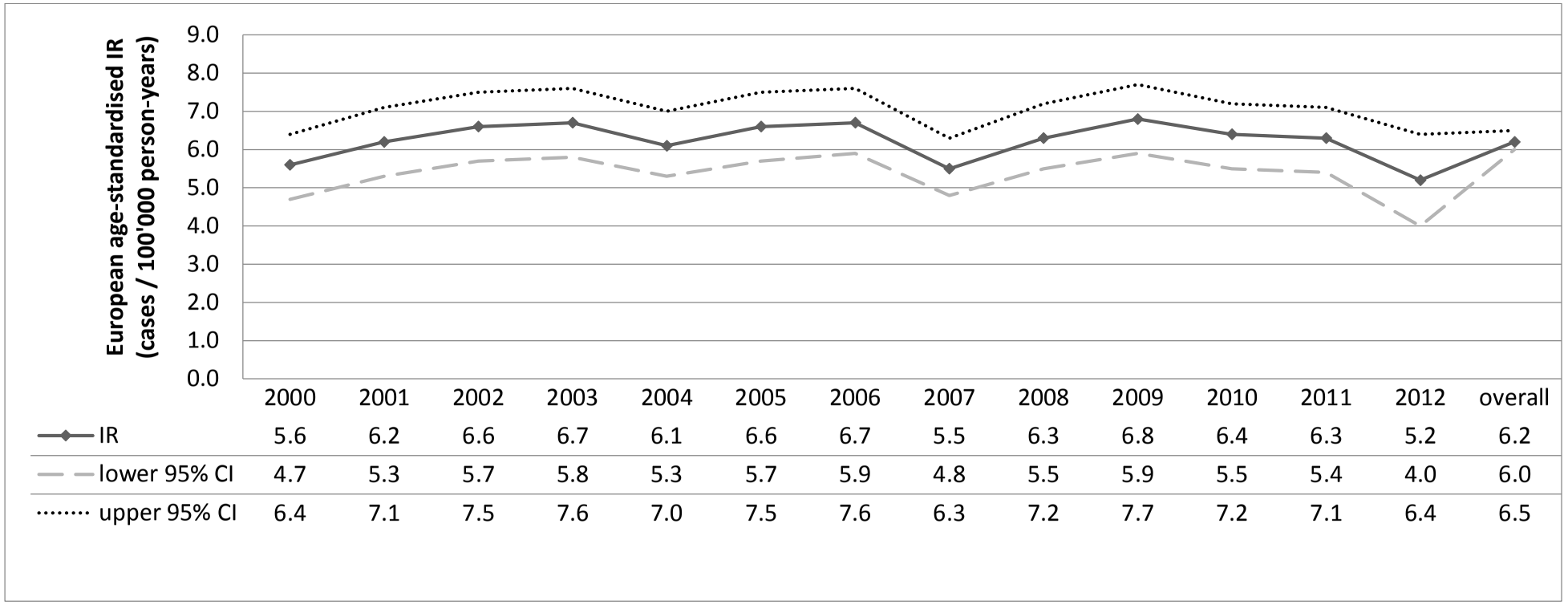
[#] including patients with lymphoma NOS, Non-Hodgkin lymphoma NOS and one patient with diffuse large B-cell lymphoma

[§] other malignancies that occurred after the diagnosis of chronic lymphocytic leukaemia

Charlson comorbidity weighted index

During follow-up, the majority of patients (n=1,425, 60.1%) in the study population had none of the comorbidities defined in the Charlson comorbidity weighted index (Table 4.1-1). Of those with comorbidities, 857 (90.5%) had a Charlson comorbidity weighted index ranging from one to three. Chronic obstructive pulmonary disease (n=189) was more common in men (n=136) than in women (n=53) (72% vs. 28%, p<0.001), as were congestive heart failure (n=95, 64 men, 31 women, 67% vs. 33%, p=0.064), and hypertension (n=406, 221 men, 185 women, 54% vs. 46%, p=0.098), whereas dementia (n=60, 28 men, 32 women) was more common in women (53% vs. 47%, p=0.068). Between 2000 and 2012, there was a significant trend of decreasing average Charlson comorbidity weighted index (-7.4%, 95% CI -9.1, -5.8%, p<0.001) per year.

Figure 4.1-2: Age-standardised incidence rates of CLL between 2000 and 2012



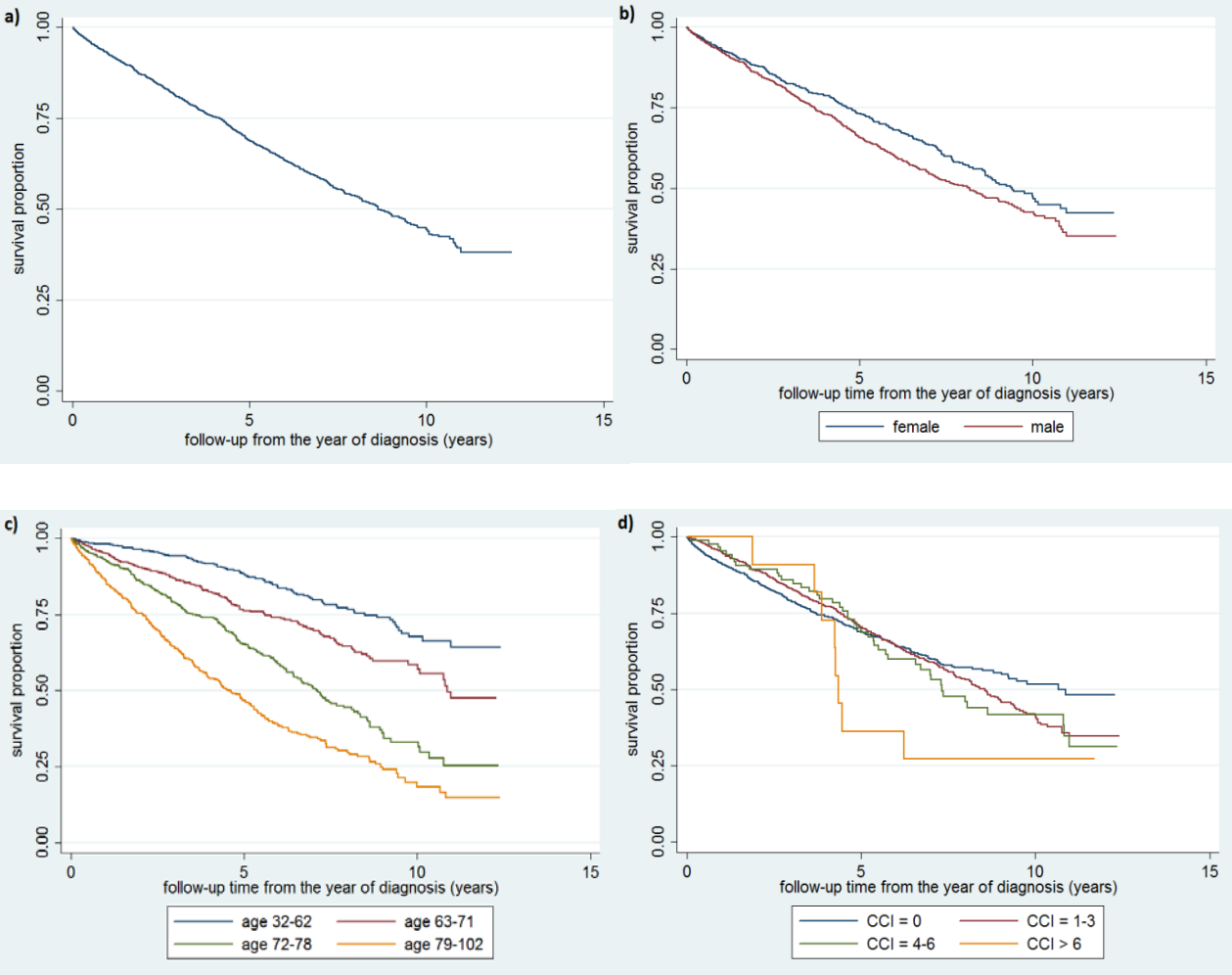
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	overall
IR	5.6	6.2	6.6	6.7	6.1	6.6	6.7	5.5	6.3	6.8	6.4	6.3	5.2	6.2
lower 95% CI	4.7	5.3	5.7	5.8	5.3	5.7	5.9	4.8	5.5	5.9	5.5	5.4	4.0	6.0
upper 95% CI	6.4	7.1	7.5	7.6	7.0	7.5	7.6	6.3	7.2	7.7	7.2	7.1	6.4	6.5

IR, incidence rate; CI, confidence interval

Follow-up and survival

Median follow-up after the diagnosis of CLL was 3.3 years (IQR 4.7 years, range: 0.02-12.4 years). About a third of all CLL patients (n=802, 31.1%) died from any cause during follow-up. The overall Kaplan-Meier estimate of median survival was 8.6 years from the year of diagnosis (Figure 4.1-3). Survival significantly (p<0.001) differed by gender, age at diagnosis, and Charlson comorbidity weighted index (Figure 4.1-3): males had a shorter survival than females (adjusted hazard ratio [HR] 1.5, 95% CI 1.3, 1.7); survival decreased per year increase in age at diagnosis (adjusted HR 1.08, 95% CI 1.07, 1.09) and decreased with one unit increase in Charlson comorbidity weighted index (adjusted HR 2.3, 95% CI 1.5, 3.6). A CLL diagnosis in the years 2001 to 2012 compared to a CLL diagnosis in 2000 was borderline significantly associated (p=0.077) with improved survival (HR 0.98, 95% CI 0.95, 1.0).

Figure 4.1-3: Kaplan-Meier estimate of overall survival



CCI, Charlson comorbidity weighted index

Kaplan-Meier estimate of a) overall survival, b) overall survival stratified by gender, c) overall survival stratified by age group and d) overall survival stratified by Charlson comorbidity weighted index

Medical resource utilisation

During a median follow-up of 3.3 (IQR 4.7) years after the diagnosis of CLL, patients had on average 74.6 GP visits, 3.2 referrals to specialists or specialised clinics, 0.5 hospitalisations and 0.1 chemotherapies or radiotherapies, as presented in detail in table 2. Prescriptions of immunosuppressants, chemotherapeutics, or antiemetics per patient per year were: 0.1 prescriptions for immunosuppressants, 0.6 prescriptions for chemotherapeutics and 1.1 prescriptions for antiemetics, respectively. Prescriptions included chemotherapeutic agents such as fludarabine (n=2), cyclophosphamide (n=3), prednisolone (n=709), and chlorambucil (n=35). No presctiption codes for monoclonal antibodies such as rituximab were recorded in our study population.

Between 2000 to 2012, the average number of recorded referrals to a specialist or specialised clinic and the average number of recorded hospitalisations per year (corrected for duration of follow-up) significantly increased by 8.1% (95% CI 6.8, 9.3%) and 16.4% (95% CI 15.4, 17.3%), respectively. In the same time period, a small increase in the number of referrals and hospitalisations per patient per year (corrected for duration of follow-up) was observed (Table 4.1-2).

In the first year following the CLL diagnosis, 0.8 referrals were recorded on average. The average number of recorded referrals was 0.5 in the second year following the CLL diagnosis, corresponding to a significant decrease of 35.3% (95% CI 28.8, 70.2%). On average, 0.13 hospitalisations were recorded in the first year following the CLL diagnosis and 0.09 hospitalisations in the second year following the CLL diagnosis. This corresponds to a significant decrease by 29.4% (95% CI 12.8, 45.9%).

Table 4.1-2: Medical resource utilisation from 2000-2012

Item	**Average number during mean follow-up of 4.1 years**	**SD**	**range**	**Average number per patient per year***	**SD**	**range**
GP visits	74.6	65.3	1-417	22.2	17.1	1-302
First visit after CLL diagnosis on average within (days)	16.5	39.3	1-1304	NA	NA	NA
In the first year following the CLL diagnosis	NA	NA	NA	17.6	13.0	1-162

Item	Average number during mean follow-up of 4.1 years	SD	range	Average number per patient per year*	SD	range
Referrals	3.2	4.4	0-65	1.0	2.4	0-41
First referral after CLL diagnosis on average within (years)	1.1	1.5	0-9.6	NA	NA	NA
In the first year following the CLL diagnosis	NA	NA	NA	0.8	1.3	0-20
Hospitalisations	0.5	1.2	0-14	0.3	2.2	0-81
First hospitalisation after CLL diagnosis on average within (yrs)	2.5	2.4	0-11.1	NA	NA	NA
In the first year following the CLL diagnosis	NA	NA	NA	0.1	0.5	0-7
Chemotherapy and Radiotherapy	0.1	0.6	0-9	0.05	0.3	0-12
First therapy after CLL diagnosis on average within (years)	2.5	2.4	0-10.4	NA	NA	NA
Blood transfusion						
First blood transfusion after CLL	0.1	1.7	0-72	0.06	0.7	0-21
diagnosis on average within (yrs)	2.9	2.8	0-9.9	NA	NA	NA
Tests and investigations						
Bone marrow examination (n=4)	1.3	0.5	1-2	-	-	-
Haemoglobin (n=2,146)	8.2	10.0	1-136	2.3	2.7	0.1-37
Neutrophil count (n=2,034)	7.5	8.8	1-112	2.1	2.6	0.1-37
Red blood cell count (n=1,953)	6.4	7.7	1-95	1.8	2.7	0.1-61
White blood cell count (n=2,137)	8.2	10.0	1-138	2.3	2.8	0.1-39
Platelets (n=2,131)	9.0	11.2	1-138	2.5	3.2	0.1-61
Prescriptions						
Chemotherapeutics	1.9	14.3	0-595	0.6	2.9	0-69
Immunsuppresives	0.4	6.8	0-297	0.1	1.1	0-35
Antiemetics	3.6	16.3	0-362	1.1	4.3	0-101
Antidepressants	5.9	19.4	0-315	1.6	4.8	0-56
Antifungals	0.7	2.9	0-55	0.2	1.2	0-20
Antibiotics	7.0	12.7	0-158	1.8	3.1	0-55
Antivirals	0.6	3.0	0-63	0.1	0.8	0-16
Growth factors	0.03	0.8	0-35	0.01	0.3	0-12

Trend analyses

Item	Estimate	95% CI	p-value
Change in average number of referrals per year	16.4%	15.4%, 17.3%	p<0.001
Change in number of referrals per patient per year	0.05%	0.04%, 0.06%	p<0.001
Change in average number of referrals in the second year compared to the first year following the CLL diagnosis	-35.3%	-28.8%, -70.2%	p<0.001
Change in average number of hospitalisations per year	8.1%	6.8%, 9.3%	p<0.001
Change in number of hospitalisations per patient per year	0.05%	0.02%, 0.08%	p=0.002

Item	Estimate	95% CI	p-value
Change in average number of hospitalisations in the second year compared to the first year following the CLL diagnosis	-29.4%	-12.8%, -45.9%	p<0.001

CI, confidence interval; CLL, chronic lymphocytic leukaemia; GP, general practitioner; NA, not applicable; SD, standard deviation; yrs, years

Where n is not specified, the number was obtained including all patients

* corrected for follow-up

Discussion

In this large epidemiological study based on primary care data from the UK CPRD, we identified 2,576 eligible adult patients with a diagnosis of CLL between 2000 and 2012**.** The overall age-standardised incidence rate was 6.2/100,000 (95% CI 6.0, 6.5/100,000) person-years. We found that CLL incidence rates in the UK CPRD increased slightly between 2000 and 2005 and then stabilised between 2006 and 2010. Male and elderly patients were more often diagnosed with CLL. More than a third of the patients had comorbidities defined in the Charlson comorbidity weighted index. Overall median survival after the CLL diagnosis was about 9 years and significantly differed by age, gender and Charlson comorbidity weighted index. The average number of recorded referrals to a specialist or specialised clinic and hospitalisations per patient per year significantly increased between 2000 and 2012. Referrals and hospitalisations were less frequent in the second year following the CLL diagnosis compared to the first year following the CLL diagnosis.

Our estimate of the crude incidence rate of CLL in the CPRD population (7.2/100,000, 95% CI 6.9, 7.4/100,000 person-years) was higher than the one reported in a Czech study (5.8 and 6.2/100,000 people in 2006 and 2007, respectively) [167]. Compared to our age-standardised incidence rate estimate of 6.2/100,000 person-years, lower cancer registry-based age-standardised incidence rates of CLL (3.8/100,000 person-years over a 20-year time period) were reported for the Netherlands [168] and for the UK (3.6/100,000 people in 2009) [169]. Our estimate was higher, most likely because haematological malignancies are more frequently recorded in the CPRD compared to cancer registries [170] and we used the new European standard population, published in 2013 [164], to calculate age-standardised incidence rates. We observed a trend of increasing CLL incidence rates with increasing year of study, although the difference did not reach statistical significance. Notably, between 2003 and 2009, annual incidence rates of all cancers in the UK also

increased [169]. An observed decline in the incidence rate between 2011 and 2012 as shown in figure 2 was likley explained by the fact that data were only availabe through June 2012.

In our study, CLL occurred more often in males and elderly patients. More than a third of the CLL patients had comorbidities as defined by the Charlson comorbidity weighted index [165]. A similar pattern was seen in a Danish study of different types of cancer including leukaemia [171]. The average Charlson comorbidity weighted index in the CPRD population significantly decreased between 2000 and 2012. This is probably because high risk patients presenting with several comorbidities are being managed in the hospital and not in primary care. Although patients are currently diagnosed at an earlier disease stage [63], the median age at CLL diagnosis is still above 70 years and a substantial proportion of the patients have one or more comorbidities [154] as defined in the Charlson comorbidity weighted index [165]. The Cumulative Illness Rating Scale (CIRS), which is often used to assess the health status in patients with CLL, could not be applied [172,173]. The severity of morbidities recorded in the CPRD was not provided for all morbidities. For example, the CPRD coding system does not provide a code that allows a straightforward differentiation between mild, moderate or severe disease in the case of mental or metabolic illnesses.

During the time period from 2000 to 2012, average medical resource utilisation per year in terms of number of recorded referrals and hospitalisations increased significantly. As our trend analyses were controlled for duration of follow-up, the changes in medical resource utilisation may reflect real changes rather than a cohort effect. But the increase could also be due to changes in the way GPs recorded these referrals and hospitalisations. The OECD Health Data 2013 [174] found that the number of GP visits reported for the general UK population decreased over time. On the other hand, key statistics of the UK NHS reported an increase in hospital admissions and outpatient appointments for the general UK population [175]. Changing clinical practice patterns may partly explain the change in medical resource utilisation. The decreasing number of referrals and hospitalisations found in the CPRD population in the second year compared to the first year following the CLL diagnosis could reflect referral back into the community for GP follow-up of low risk patients (i.e. to monitor disease progression). An increase of referrals and hospitalisations in the CPRD population could reflect need for immediate treatment after initial CLL diagnosis.

To our knowledge, this is the first study to describe population-based incidence rates and trends in medical resource utilisation in CLL patients using UK-based primary care data.

Previous literature suggests that most of the diagnoses coded in the CPRD are accurately recorded and agree with other databases and national statistics [176], and cancer registries [170]. The validity of the CLL diagnosis was further assessed by screening random records of 100 CLL patients for CLL-concomitant laboratory measures, diagnostic tests and symptoms. The CLL diagnosis was considered validated if the patient record included at least one screening criterion for CLL. Only patients with at least three years of recorded history in the CPRD prior to the CLL diagnosis were included in our analysis to increase the probability of including only incident rather than prevalent cases Therefore, we are confident that the estimated incidence rates are reliable and generalisable to the UK population.

This study has several limitations that should be considered in interpreting our findings. Although about 70% of the CLL cases are diagnosed in asymptomatic patients [63] when they visit the GP for a routine blood count, some CLL cases may remain undetected because there is no visit to the GP for a routine blood count. Detection bias may limit the interpretation of our observed gender differences, as women may see the GP more often than men [177]. Total medical resource utilisation of CLL patients was incompletely assessed because details about secondary and tertiary care are not consistently recorded in the CPRD. Therefore, the recorded number of referrals and hospitalisations in the CPRD may be an underestimate. Details about type of chemotherapy regimen, for example, are often lacking as few chemotherapies are administered in the ambulatory setting.

Medical records of GPs are a valuable source to estimate population-based CLL incidences and trends in incidences in the UK. Further research to assess details of medical resource utilisation in secondary and tertiary care, especially regarding chemotherapy use, is needed to better understand total medical resource utilisation and treatment patterns in CLL.

Conclusion

Consistent with previous literature, we found that CLL incidence rates in the UK increased over time. However, in the past five years, we observed a stabilisation of CLL incidence rates in the CPRD population, which has not been previously reported in the literature. Further research to determine CLL-specific mortality and resource utilisation in non-primary care is needed as cancer patients are often treated in hospitals.

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Author contributions

All authors were involved in the design of the study. A.M.P. was responsible for the first draft of the protocol, which was critically reviewed and approved by all authors. A.M.P was responsible for data analysis and the first draft of the manuscript. All authors contributed to data interpretation, critically reviewed all manuscript versions and read and agreed upon the final version of the manuscript.

Conflict of interest

No financial support was received for the conduct of this study. All authors declare no conflicts of interest related to this article.

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4.2 Multivariable regression analysis of febrile neutropenia occurrence in early breast cancer patients receiving chemotherapy assessing patient-related, chemotherapy-related and genetic risk factors

Alena M. Pfeil[1*], Christof Vulsteke[2*], Robert Paridaens[2], Anne-Sophie Dieudonné[3,5], Ruth Pettengell[4], Sigrid Hatse[2], Patrick Neven[5], Diether Lambrechts[6,7], Thomas D. Szucs[1], Matthias Schwenkglenks[1§], Hans Wildiers[2,5§#]

*These authors contributed equally to this work
[§]Joint last authorship

1 Institute of Pharmaceutical Medicine (ECPM), University of Basel, Basel, Switzerland

2 Laboratory of Experimental Oncology (LEO), Department of Oncology, KU Leuven, and Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Belgium

3 Department of Oncology, KU Leuven, Leuven, Belgium

4 Cellular and Molecular Medicine, St. George's University of London, London, UK

5 Multidisciplinary Breast Center, University Hospitals Leuven, KU Leuven, Leuven, Belgium

6 Vesalius Research Center, Vlaams Instituut voor Biotechnologie (VIB), Flanders

7 Laboratory for Translational Genetics, Department of Oncology, KU Leuven, Leuven, Belgium

Corresponding author:
Prof. Dr. Hans Wildiers, Multidisciplinary Breast Center, University Hospitals Leuven, KU Leuven, Leuven, Belgium, Email: hans.wildiers@uzleuven.be

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Abstract

Background: Febrile neutropenia (FN) is common in breast cancer patients undergoing chemotherapy. Risk factors for FN have been reported, but risk models including genetic variability have yet to be described. This study aimed to evaluate the predictive value of patient-related, chemotherapy-related and genetic risk factors.

Methods: Data from consecutive breast cancer patients receiving chemotherapy with 4-6 cycles of fluorouracil, epirubicin and cyclophosphamide (FEC) or 3 cycles of FEC and docetaxel were retrospectively recorded. Multivariable logistic regression was carried out to assess risk of FN during FEC chemotherapy cycles.

Results: Overall, 166 (16.7%) out of 994 patients developed FN. Significant risk factors for FN in any cycle and the first cycle were lower platelet count (OR=0.78 [0.65;0.93]) and haemoglobin (OR=0.81 [0.67;0.98]) and homozygous carriers of the rs4148350 variant T-allele (6.7 [1.04;43.17]) in *MRP1*. Other significant factors for FN in any cycle were higher alanine aminotransferase (OR=1.02 [1.01;1.03]), carriers of the rs246221 variant C-allele (OR=2.0 [1.03;3.86]) in *MRP1* and the rs351855 variant C-allele (OR=2.48 [1.13;5.44]) in *FGFR4*. Lower height (OR=0.62 [0.41;0.92]) increased risk for FN in cycle 1.

Conclusions: Both established clinical risk factors and genetic factors predicted FN in breast cancer patients. Prediction was improved by adding genetic information but overall remained limited. Internal validity was satisfactory. Further independent validation is required to confirm these findings.

Background

Chemotherapy-induced neutropenia (CIN) and febrile neutropenia (FN) are serious and frequent complications in patients with breast cancer receiving adjuvant chemotherapy, and they result in hospitalisations [101,178,179] and chemotherapy dose reductions or delays that impact on treatment outcome and short-term mortality [102]. Adjuvant fluorouracil, epirubicin, and cyclophosphamide (FEC) chemotherapy has an FN risk of between 9% and 14% (low-intermediate risk) [98].

Antibacterial or antifungal prophylaxis has recently been recommended for neutropenic patients expected to have a prolonged low neutrophil count or with other risk factors that favour complications [144]. Prophylaxis with granulocyte colony-stimulating factor (GCSF) in patients at high risk of FN (>20%) is recommended in international guidelines [98,141,142]. For chemotherapy regimens with an intermediate FN risk (10-20%), the European Organisation for Research and Treatment of Cancer (EORTC) G-CSF guideline recommends that patient risk factors should also be considered to determine individual risk of FN [98] and the likely benefit of prophylactic GCSF. Therefore, it is important to identify patients at high risk of FN before the initiation of chemotherapy to provide them with appropriate prophylactic measures.

Risk models for the occurrence of CIN [106] and FN [104] in patients with breast cancer have been published. The risk factors identified included: older age, lower weight, higher planned dose of chemotherapy, higher number of planned chemotherapy cycles, vascular comorbidity, lower baseline white blood cell count (WBC), lower platelet and neutrophil count and higher baseline bilirubin. Prior chemotherapy, abnormal liver or renal function, low WBC, higher chemotherapy intensity and planned delivery were identified as risk factors for neutropenic complications in a prospective US study of patients with different types of cancer [130]. Poor performance status and low lymphocyte and neutrophil counts were risk factors in a European study of solid tumour patients [131], as were tumour stage and number of comorbidities in elderly patients with solid tumours [113].

These risk models of CIN or FN that included patient- or chemotherapy-related factors were reported to be predictive. However, more refined models are necessary to achieve satisfactory performance in independent patient populations that include existing and emerging types of data, including stable genetic factors that are easily measurable, objective, and potentially independent from the inherent viabilities of clinical decision-making. Several studies have assessed the impact of genetic factors on haematological

toxicity, but these studies were small in size or limited to only a few candidate genetic factors [122,124,125].

The objective of this study was to develop risk models for the occurrence of FN in breast cancer patients receiving FEC chemotherapy in any cycle and the first cycle based on a large set of patient-related, chemotherapy-related, and genetic characteristics.

Methods

Study population

We retrospectively studied early (i.e., no distant metastases; Stage I-IIIC) breast cancer patients treated between 2000 and 2010 at the Leuven Multidisciplinary Breast Cancer Center of the University Hospitals Leuven, Belgium. Consecutive patients were included if they received either three cycles of neoadjuvant or adjuvant combination chemotherapy consisting of FEC followed by three cycles of docetaxel or four to six cycles of FEC. Patient-related factors (genetics and tumour characteristics) and chemotherapy-related factors were retrospectively recorded in a clinical database. Haematological toxicities included were: FN (defined as an absolute neutrophil count (ANC) < 0.5 x 10^9/L and a body temperature ≥ 38°C according to the Infectious Diseases Society of America), prolonged grade 4 neutropenia (≥ 5 days), deep neutropenia (< 100/µl), grade 3/4 thrombocytopenia, and grade 3/4 anaemia during FEC chemotherapy cycles. Haematological toxicities that occurred during chemotherapy cycles with docetaxel were not included in the model. Grade 3/4 non-haematological toxicities were also recorded (toxicity grade based on the Common Terminology Criteria for Adverse Events 3.0 [180]). During most of the study period, only primary prevention with GCSF was reimbursed and, therefore, only used in selected patients aged 65 or over. Similarly, secondary use of GCSF was only reimbursed and used if patients had FN in the previous cycle or if deep neutropenia occurred for at least five days (although the latter was not systematically measured during the study period).

The study design and full analysis of single nucleotide polymorphisms (SNPs) have previously been described in detail [126]; however, in the previous analysis the association of SNPs with FN was only adjusted for age, growth factor use, BMI, and planned cycles of chemotherapy. Only those SNPs that have been reported to be associated with haematological toxicity or to play a role in the metabolism of FEC chemotherapy were included in the current study. Logistic regression was performed to describe the

association of SNPs with haematological toxicity, adjusted for known predictors of FN risk such as age, growth factor use, and planned number of cycles of chemotherapy. The ethics committee of the University Hospitals Leuven approved the study and all patients included in the study had given written informed consent for collection of genetic samples and for further analyses using this material and associated data.

Endpoints and predictor variables

The primary endpoint of the study was FN in any cycle, and FN occurring in the first cycle (cycle 1) was the secondary endpoint. The following variables were considered as predictors of FN: planned doses of fluorouracil, epirubicin and cyclophosphamide (FC, 600 mg/m^2 until August 2004 and 500 mg/m^2 after this date; epirubicin 100 mg/m^2), age at diagnosis, height, weight, body mass index (BMI), body surface area (BSA), chemotherapy setting (i.e. adjuvant or neoadjuvant), use of GCSF (information only available on primary or secondary use), planned cycles of FEC chemotherapy, selected SNPs [126], baseline WBC, ANC and platelet count, and other baseline laboratory parameters such as haemoglobin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine. Although timing and reasoning of GCSF use were incomplete, its potential impact on the variables included in the final model was assessed for exploratory analysis.

Statistical analysis

All analyses were performed using Stata/SE version 12.1 (StataCorp LP, College Station, TX, USA). All statistical tests were carried out two-sided at a 5% significance level and 95% confidence intervals (CIs) were obtained.

Descriptive and univariable analysis

Binary and categorical data were summarised using frequencies and percentages. Continuous data were reported using means and standard deviations. In the univariable analysis of SNPs, the impact of multiple testing was assessed by separately calculating the false discovery rate (FDR) for each endpoint [181]. Associations between the endpoints and binary or categorical variables were assessed using the chi-squared test or Fisher's exact test, as appropriate. Continuous variables and their associations with the endpoints were assessed using univariable logistic regression analysis. Variables were further assessed in multivariable logistic regression analysis if a trend was seen in the univariable analysis ($p \leq 0.25$), as recommended [182]. Linear correlation between potential predictors were assessed by calculating Pearson's correlation coefficient and monotonic correlations were assessed using Spearman's rank correlation coefficient. Variables were regarded as

being dependent if the correlation coefficient was ≥ 0.7 or the correlation p-value was ≤ 0.05.

Multivariable analysis

Multivariable logistic regression analysis was used to assess the joint explanatory value of the candidate variables identified in univariable analysis; variables were included in the final multivariable models if their corresponding *p*-value was ≤ 0.05. Where simultaneous inclusion of dependent variables led to estimation problems (collinearity issues), the variable that explained more of the variability present in the endpoint was finally used. As patient-related and chemotherapy-related factors were already established as risk factors in several previous risk models, these variables were entered into the model first, ordered according to the *p*-value obtained in univariable analysis. SNPs were subsequently added. Interactions between variables were assessed. Model fit was assessed with the Hosmer-Lemeshow [183] goodness-of-fit test. Test characteristics such as specificity (proportion of negatives correctly identified as not having an event), sensitivity (proportion of positives correctly identified as having an event), positive predictive value (PPV, proportion of patients identified to have an event who had an event) and negative predictive value (NPV, proportion of patients identified not to have an event who did not have an event) were obtained. The predictive ability of the final models was assessed by calculating the area under the receiver operating characteristic (ROC; sensitivity over 1-specificity) curve.

To test the internal validity of the final models, nonparametric bootstrapping was performed [184]. Bootstrap estimates of the 95% CIs of the multivariable models were obtained by resampling the data 200 times. The obtained 95% CI estimates of the bootstrap resampling were compared to the 95% CIs calculated by the multivariable logistic regression model.

Results

Characteristics of the study group

Of 1,012 patients that received FEC chemotherapy between 2000 and 2010, 18 patients were excluded due to receiving chemotherapy prior to FEC, which may have impacted on FN risk. The majority of 994 eligible patients received adjuvant chemotherapy (n = 874, 88.0%); the remainder received neoadjuvant chemotherapy. Most patients received three cycles of combination chemotherapy with FEC followed by three cycles of docetaxel (n = 507, 51.0%) or six cycles of FEC (n = 405, 40.7%) (Table 4.2-1). The most common type of breast cancer was invasive ductal carcinoma (n = 823, 82.8%) and patients mostly had

grade 2 (n = 334, 34.1%) or grade 3 (n = 606, 61.9%) tumours. FN occurred in any cycle in 166 (16.7%) patients, of which 107 (10.8%) had FN in the first cycle of FEC chemotherapy. The most common haematological toxicity was prolonged grade 4 neutropenia (n = 345, 34.7%). Other haematological toxicities such as grade 3/4 thrombocytopenia and severe bleeding, and grade 3/4 non-haematological toxicities such as diarrhoea, mucositis, and neuropathy were rare (n < 10, <1%). Primary prophylactic GCSF (before a CIN or FN event occurred) was given to 15 (1.5%) patients and the majority received no GCSF (n = 654, 65.8%). Additional toxicities and other relevant characteristics such as planned number of chemotherapy cycles, tumour stage, and subtype are presented in Table 4.2-1. The list of SNPs included in the analyses is shown in Table 4.2-2.

Table 4.2-1: Characteristics of the study population, the tumours, and the administered chemotherapy including toxicities

Patient characteristics	**Mean ± standard deviation or frequency (%)**
Age at diagnosis (years) (*n* = 994)	50.4 ± 9.6
Body mass index (kg/m^2) (*n* = 981)	24.9 ± 4.1
Body surface area (m^2) (*n* = 993)	1.7 ± 0.1
Tumour characteristics	
Tumor Stadium	994 (100)
Primary tumour	966 (97.2)
Relapsed tumour	28 (2.8)
Tumour grade[a]	979 (98.5)
1	39 (4.0)
2	334 (34.1)
3	606 (61.9)
Tumour type	994 (100)
Invasive ductal carcinoma	823 (82.8)
Invasive lobular carcinoma	103 (10.4)
mixed	27 (2.7)
others	41 (4.1)
Tumour stage[b]	978 (98.4)
I	113 (11.5)
IIA	306 (31.3)
IIB	245 (25.1)
IIIA	193 (19.7)
IIIB	44 (4.5)
IIIC	77 (7.9)
Receptor status	
estrogen receptor positive	683 (68.8)
progesterone receptor positive	577 (58.1)

Tumour characteristics	
Receptor status	
HER2 positive	205 (20.7)
Subtype[c]	981 (98.7)
luminalA	325 (33.1)
luminalB HER2-	234 (23.9)
luminalB HER2+	121 (12.3)
HER2-like	84 (8.6)
triple negative	217 (22.1)
Nottingham Prognostic Index (NPI)[d] (*n* = 757)	5.0 ± 0.9
Chemotherapy characteristics	
Chemotherapy setting	994 (100)
adjuvant	874 (87.9)
neoadjuvant	120 (12.1)
Planned cycles of FEC chemotherapy	994 (100)
3 cycles FEC	559 (56.2)
Chemotherapy characteristics	
Planned cycles of FEC chemotherapy	
4 or 5 cycles FEC	2 (0.2)
6 cycles FEC	433 (43.6)
Relative dose intensity (RDI) (*n* = 994)	0.96 ± 0.1
Growth factor use	994 (100)
primary	15 (1.5)
secondary	325 (32.7)
none	654 (65.8)
Baseline laboratory parameters	
White blood cell count (10^9/L) (*n* = 985)	7.2 ± 2.0
Absolute neutrophil count (10^9/L) (*n* = 937)	4.4 ± 1.6
Haemoglobin (g/dl) (*n* = 989)	13.3 ± 1.0
Platelets (10^9/L) (*n* = 985)	275.4 ± 65.1
Total bilirubin (mg/dl) (*n* = 915)	0.4 ± 0.2
Creatinine (mg/dl) (*n* = 957)	0.8 ± 0.1
Alanine aminotransferase (U/L) (*n* = 955)	23.3 ± 15.3
Aspartate aminotransferase (U/L) (*n* = 955)	21.9 ± 11.1
FEC chemotherapy toxicities	
Febrile neutropenia	166 (16.7)
- Febrile neutropenia in first cycle	107 (10.7)
Prolonged (≥ 5 days) grade 4 neutropenia	345 (34.7)
Deep neutropenia (< 100/µl)	93 (9.4)
Other grade 3-4 toxicities	46 (4.6)

FEC, fluorouracil, epirubicin and cyclophosphamide; HER2, human epidermal growth factor receptor 2

[a] according to the Ellis and Elston grading system [185]

[b] according to the TNM classification [186]

[c] according to Brouckaert et al. [187]

[d] according to Lee et al. [188]

Table 4.2-2: List of included single nucleotide polymorphisms (SNPs), and their frequencies (percentages)

	Genotype					
Gene	**n**	**GG** **n (%)**	**GA** **n (%)**	**AA** **n (%)**	**CC** **n (%)**	**CA** **n (%)**
ABCC2/MRP2rs8187710	954	842 (88.3)	110 (11.5)	2 (0.2)		
ABCG2/BRCPrs2231137	955	888 (93.0)	67 (7.0)			
CYP2B6rs2279343	910	57 (6.2)	382 (42.0)	471 (51.8)		
CYP2C8rs72558196	960			960 (100)		
CYP2C9rs1057910	954			853 (89.4)	3 (0.3)	98 (10.3)
CYP2C19rs4244285	946	652 (68.9)	266 (28.1)	28 (3.0)		
CYP2C19rs4986893	960	960 (100)				
CYP3A4rs2740574	955	2 (0.2)	57 (6)	896 (93.8)		
CYP3A4rs55785340	957			957 (100)		
CYP3A5rs776746	959	834 (87.0)	118 (12.3)	7 (0.7)		
DPYDrs1801159	960	47 (4.9)	267 (27.8)	646 (67.3)		
DPYDrs3918290	949	945 (99.6)	4 (0.4)			
DPYDrs1801160	957	853 (89.1)	96 (10.0)	8 (0.9)		
GSTA1rs3957357	938	329 (35.1)	441 (47.0)	168 (17.9)		
GSTP1rs1695	959	118 (12.3)	452 (47.1)	389 (40.6)		
MRP1rs1883112	956	295 (30.9)	485 (50.7)	176 (18.4)		
MRP1rs7853758	952	701 (73.6)	231 (24.3)	20 (2.1)		
MTHFRrs1801131	951			446 (46.9)	92 (9.7)	413 (43.4)
UGT2B7rs12233719	949	949 (100)				
UGT2B7rs7662029	955	210 (22.0)	473 (49.5)	272 (28.5)		
XPD/ERCC2rs1799793	954	412 (43.2)	429 (45.0)	113 (11.8)		
XRCC1rs25489	954	875 (91.7)	77 (8.1)	2 (0.2)		
XRCC3rs861534	949	357 (37.6)	441 (46.5)	151 (15.9)		
Gene	**n**	**TT** **n (%)**	**CC** **n (%)**	**CT** **n (%)**	**AA** **n (%)**	**TA** **n (%)**
ABCC2/MRP2rs17222723	951	843 (88.6)			2 (0.2)	106 (11.2)
ABCC2/MRP2rs2804402	935	297 (31.8)	185 (19.8)	453 (48.4)		
CYP2B6rs8192709	927		846 (91.3)	87 (8.7)		
CYP2C8rs10509681	960	740 (77.1)	12 (1.2)	208 (21.7)		
CYP2C9rs1799853	957	15 (1.6)	712 (74.4)	230 (24)		
CYP3A4rs4986910	959	938 (97.8)		21 (2.2)		
DPYDrs1801265	960	635 (66.1)	46 (4.8)	279 (29.1)		
Gene	**n**	**TT** **n (%)**	**CC** **n (%)**	**CT** **n (%)**	**AA** **n (%)**	**TA** **n (%)**
FGFR4rs351855	954	88 (9.2)	461 (48.3)	405 (42.5)		
GSTP1rs1138272	952	6 (0.6)	778 (81.7)	168 (17.7)		
MDRI/ABCB1rs1045642	914	265 (29.0)	208 (22.8)	441 (48.2)		
MRP1rs13058338	949	482 (50.8)			67 (7.1)	400 (42.1)
MRP1rs246221	956	462 (48.3)	71 (7.4)	423 (44.3)		

Gene	n	TT n (%)	CC n (%)	CT n (%)	AA n (%)	TA n (%)
MRP1rs3743527	930	13 (1.4)	562 (60.4)	355 (38.2)		
MRP1rs4673	954	115 (12.0)	406 (42.6)	433 (45.4)		
MTHFRrs1801133	959	121 (12.6)	401 (41.8)	437 (45.6)		
NQO1rs1800566	958	35 (3.6)	605 (63.2)	318 (33.2)		
UGT2B7rs7439366	955	272 (28.5)	210 (22.0)	473 (49.5)		
UGT2B7rs7668282	954	940 (98.5)	1 (0.1)	13 (1.4)		
Gene	**n**	**GG** **n (%)**	**GT** **n (%)**	**TT** **n (%)**	**CC** **n (%)**	**CG** **n (%)**
ALDH3A1rs2228100	934	67 (7.2)			554 (59.3)	313 (33.5)
CYP2B6rs3745274	954	535 (56.1)	365 (38.2)	54 (5.7)		
GPX4rs757229	940	263 (28.0)			212 (22.5)	465 (49.5)
MRP1rs4148350	957	847 (88.5)	105 (11.0)	5 (0.5)		
MRP1rs45511401	960	847 (88.2)	109 (11.4)	4 (0.4)		
UGT2B7rs3924194	954	19 (2.0%)			712 (74.6)	223 (23.4)
XPD/ERCC2rs13181	951	116 (12.2)	449 (47.2)	386 (40.6)		
Gene	**n**	**GG** **n (%)**	**GT** **n (%)**	**TT** **n (%)**	**GA** **n (%)**	**TA** **n (%)**
MDRI/ABCB1rs2032582	948	283 (29.9)	445 (46.9)	185 (19.5)	23 (2.4)	12 (1.3)
TYMSrs11280056	918	AAGTTA 442 (48.2)		AAGTTA.DEL 394 (42.9)		DEL 82 (8.9)

Univariable analysis

All candidate predictors ($p \leq 0.25$) for FN in any cycle and in cycle 1 are shown in Table 4.2-3. Patient-related factors (genetics, laboratory parameters, etc.) and chemotherapy-related factors fulfilled the inclusion criteria for the multivariable analysis. The number of planned FEC cycles, WBC, ANC, platelet count, and haemoglobin were significantly associated with FN in any cycle and cycle 1 ($p \leq 0.5$). SNPs significantly associated with FN in any cycle and cycle 1 were the rs4148350, rs45511401, and rs246221 variants in *MRP1* (multidrug resistance-associated protein 1). The FDR for associated SNPs for any cycle FN was 0.47 and 0.33 for cycle 1 FN. There were no correlations between SNPs included in the final model and patient-related or chemotherapy-related factors.

Risk factors of febrile neutropenia in any cycle

Multivariable regression identified the following factors to be significantly associated with a higher occurrence of FN: lower platelet count and lower haemoglobin at baseline, higher ALT, and the following SNPs: rs4148350 and rs246221 in *MRP1* and rs351855 in *FGFR4* (fibroblast growth factor receptor 4) (Table 4.2-4). Homozygous carriers of the rs4148350

T-allele had a higher risk of FN than carriers of the homozygous or heterozygous G-allele (FN risk of 80% versus 15% or 25%). For rs246221, homozygous carriers of the T-allele variant had a lower risk of FN than carriers with at least one C-allele (FN risk of 13% versus 20% or 24%). Patients with the TT genotype of rs351855 were protected against FN compared to patients carrying at least one C-allele (FN risk of 10% versus 19% or 16%).

Table 4.2-3: Candidate predictors from univariable analysis

	FN in any cycle		**FN in cycle 1**	
Variable	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Platelets (10^9/L, per 50 units change)	0.80 (0.69; 0.92)	0.002	0.78 (0.66; 0.93)	0.005
ANC (10^9/L)	0.87 (0.77; 0.98)	0.023	0.86 (0.74; 1.00)	0.046
ALT (U/L)	1.01 (1.00; 1.02)	0.024	-	-
WBC (10^9/L)	0.90 (0.83; 0.99)	0.032	0.88 (0.79; 0.99)	0.028
Height (cm)	-	-	1.03 (1.00; 1.07)	0.043
Haemoglobin (g/dl)	0.87 (0.73; 1.02)	0.094	0.80 (0.66; 0.98)	0.030
Planned cycles FEC (6 vs. 3 cycles)	1.09 (0.98; 1.22)	0.129	-	-
AST[a] (U/l)	1.00 (0.99; 1.02)	0.210	-	-
BSA (m^2)	-	-	2.44 (0.59; 10.03)	0.217
Creatinin (mg/dl)	2.04 (0.66; 6.33)	0.219	-	-
Planned dose of epirubicin (100mg/m^2)	-	-	1.01 (0.99; 1.02)	0.217
Single nucleotide polymorphisms	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
MRP1rs4148350		0.000		0.004
-GT vs. GG	1.82 (1.12; 2.94)	0.015	2.09 (1.21; 3.61)	0.008
-TT vs. GG	22.06 (2.45; 198.96)	0.006	6.30 (1.04; 38.28)	0.045
MRP1rs45511401[b]		0.000		0.004
-GT vs. GG	1.80 (1.12; 2.89)	0.015	1.82 (1.05; 3.17)	0.034
-TT vs. GG	16.40 (1.69; 158.84)	0.016	9.20 (1.28; 66.20)	0.027
MRP1rs246221		0.004		0.039
-TT vs. CC	0.47 (0.25; 0.86)	0.014	0.49 (0.24; 1.00)	0.053
-TC vs. CC	0.80 (0.44; 1.45)	0.459	0.80 (0.40; 1.61)	0.530
FGFR4rs351855		0.098	-	-
-CT vs. CC	1.25 (0.88; 1.77)	0.216		
-TT vs. CC	0.60 (0.29; 1.24)	0.166		
CYP3A4rs4986910		0.171	-	-
-TC vs. TT	0.24 (0.03; 1.84)			
XRCC3rs861534		0.130		0.044
-GG vs. AA	1.25 (0.76; 2.07)	0.381	1.73 (0.91; 3.29)	0.095
-GA vs. AA	0.86 (0.52; 1.42)	0.544	1.03 (0.53; 1.99)	0.930

Single nucleotide polymorphism	OR (95% CI)	p-value	OR (95% CI)	p-value
TYMSrs11280056		0.114	-	-
AAGTTA.DEL vs. AAGTTA	0.88 (0.60; 1.27)	0.486		
DEL vs. AAGTTA				
	1.60 (0.91; 2.82)	0.100		
GSTP1rs1695		0.228	-	-
-AG vs. AA	0.75 (0.53; 1.08)	0.124		
-GG vs. AA	0.70 (0.40; 1.25)	0.231		
GSTA1rs3957357	-	-		0.163
-GG vs. AA			0.95 (0.49; 1.83)	0.875
-GA vs. AA			1.45 (0.80; 2.65)	0.223
ALDH3A1rs2228100	-	-		0.188
-GG vs. CC			1.86 (0.92; 3.76)	0.086
-GC vs. CC			1.27 (0.81; 1.98)	0.297
MRP1rs1883112	-	-		0.187
-AG vs. AA			0.87 (0.52; 1.46)	0.594
-GG vs. AA			0.59 (0.32; 1.08)	0.087
UGT2B7rs7439366	-	-		0.204
-TT vs. CC			1.08 (0.57; 2.04)	0.813
-TC vs. CC			1.52 (0.87; 2.65)	0.139
UGT2B7rs7662029	-	-		0.204
-GG vs. AA			0.93 (0.49; 1.75)	0.813
-GA vs. AA			1.41 (0.86; 2.31)	0.174

ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; CI, confidence interval; FEC, fluorouracil, epirubicin and cyclophosphamide; FN, febrile neutropenia; WBC, white blood cell count

Odds ratios and 95% confidence intervals are reported per 1 unit change if not otherwise indicated

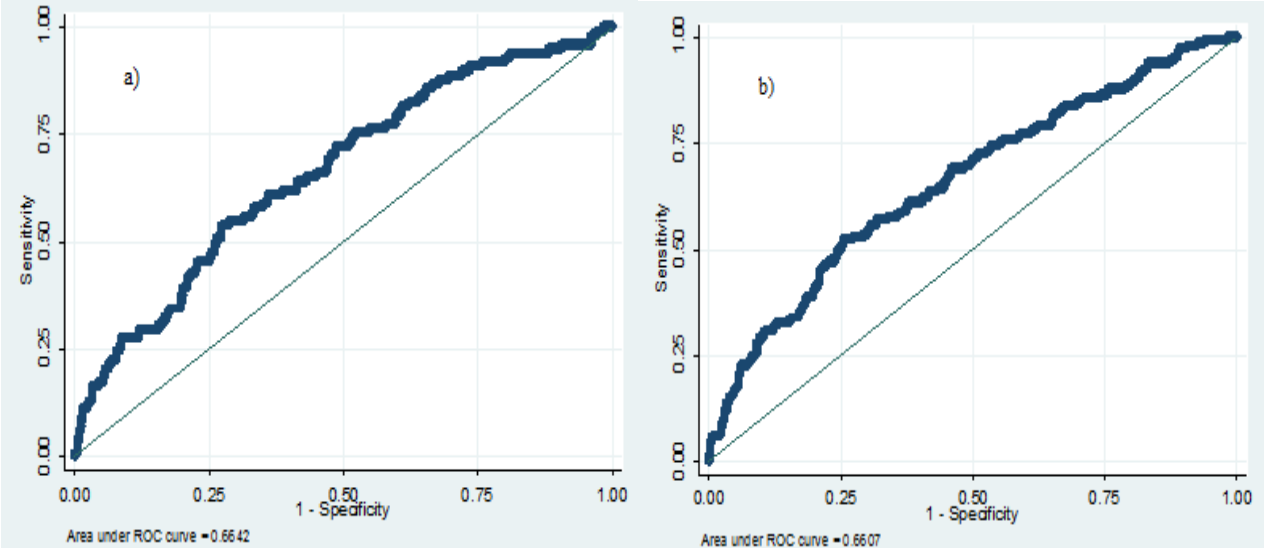
[a] highly correlated with alanine aminotransferase (Pearson's correlation coefficient 0.76) and not included in multivariable analysis

[b] highly correlated with MRP1rs4148350 (Spearman correlation coefficient 0.81) and not included in multivariable analysis

The area under the ROC curve was 0.661 (CI 0.629-0.691), as shown in Figure 4.2-1: a value of 1 would denote perfect discrimination and 0.5 discrimination no better than chance. Overall, 864 of 910 patients (84.0%) were correctly classified by the logistic regression model at a predicted probability cut-off of 0.5; six out of 150 having FN and 758 out of 760 not having FN. Sensitivity was very low (4.0%) compared to specificity (99.7%). NPV and PPV were similar; the proportion of patients correctly identified not to have FN was 84.0% and the proportion of patients correctly identified to have FN was 75.0%. When the optimal cut-off of the model was used (i.e., predicted probability of 0.1609, where sensitivity and specificity were almost identical at 61.3%), the model correctly classified

61.2% of the patients and PPV and NPV were 23.8% and 88.9%, respectively.

Figure 4.2-1: Receiver operating characteristic curves



Receiver operating curve (ROC) for a) FN in any cycle and b) FN in cycle 1

Bisecting line indicates a predictive ability that is no better than chance (ROC = 0.5)

Internal validity of the FN in any cycle model was satisfactory; the 95% CIs of the bootstrap resampling were similar to the 95% CIs calculated by the multivariable logistic regression model.

Risk factors of febrile neutropenia in cycle 1

Lower platelet count, haemoglobin at baseline and lower patient height were significantly associated with a higher risk of FN in cycle 1 (Table 4.2-4). The SNP found to be significantly associated with FN in cycle 1 was rs4148350 in *MRP1*. For rs4148350, homozygous carriers of the T-allele had a higher risk of FN in cycle 1 than carriers of the homozygous or heterozygous G-allele (FN risk of 40% versus 10% or 18%). We found a statistically significant interaction between haemoglobin and height that increased the protective effect of higher haemoglobin and increased height but did not affect the other main effects of the model.

The area under the ROC curve was 0.664 (CI 0.633-0.694) as presented in Figure 4.2-1. At a probability cut-off of 0.5, one out of 98 patients was correctly classified having FN in cycle 1 and all 839 patients without FN in cycle 1 were correctly classified not having FN (overall, 89.7% correct classifications). Sensitivity was very low (1.0%); specificity was 100%, PPV was 100%, and NPV was 89.6%. At the optimal probability cut-off for the model (0.1041), 61.5% of the patients were correctly classified, sensitivity and specificity were

61%, PPV was 15.7%, and NPV was 93.1%. The 95% CIs of the bootstrap resampling were similar to the 95% CIs calculated by the multivariable logistic regression model, which supports the internal validity of the FN in the first cycle model.

Table 4.2-4: Logistic regression models for febrile neutropenia occurrence in any cycle and the first cycle of chemotherapy

Determinant	**FN in any cycle (*n* = 910)**		**FN in cycle 1 (*n* = 937)**	
	Odds ratio (95% CI)	***p*-value**	**Odds ratio (95% CI)**	***p*-value**
Platelets (10^9/L, per 50 units change)	0.780 (0.671; 0.906)	0.001	0.777 (0.649; 0.929)	0.006
HB (g/dl, per 1 unit change)	0.812 (0.673; 0.978)	0.029	0.001 (<0.001; 0.194)	0.009
Height (cm, per 1 unit change)	-	-	0.617 (0.414; 0.919)	0.018
Interaction (height and HB)[a]	-	-	1.040 (1.008; 1.072)	0.012
ALT (U/L, per 1 unit change)	1.016 (1.005; 1.027)	0.003	-	-
MRP1rs4148350 - GT[b] vs. GG - TT[c] vs. GG	 1.494 (0.890; 2.507) 17.13 (1.72; 170.90)	0.019 0.129 0.016	 2.149 (1.226; 3.768) 6.696 (1.039; 43.167)	0.006 0.008 0.046
MRP1rs246221 - TT[d] vs. CC - TC vs. CC	 0.501 (0.259; 0.969) 0.805 (0.423; 1.533)	0.023 0.040 0.510	-	-
FGFR4rs351855 - CT vs. CC - TT[e] vs. CC	 1.253 (0.862; 1.821) 0.505 (0.230; 1.113)	0.062 0.238 0.090	-	-

ALT, alanine aminotransferase; CI, Confidence interval; FN, febrile neutropenia; HB, haemoglobin

[a] did not affect the odds ratio of the other main effects of the regression model

[b] 105/957 (11.0%) patients are carriers of the GT genotype and 19 (18.1%) out of those 105 patients had febrile neutropenia in cycle 1 of chemotherapy

[c] 5/957 (0.5%) patients are homozygous carriers of the T-allele and 4 (80%) out of those 5 patients had febrile neutropenia in any cycle of chemotherapy and 2 (40%) had febrile neutropenia in cycle 1

[d] 462/956 (48.3%) patients are homozygous carriers of the T-allele and 59 (12.8%) out of those 462 patients had febrile neutropenia in any cycle of chemotherapy

[e] 88/954 (9.2%) patients are homozygous carriers ot the T-allele and 9 (10.2%) out of those 88 patients had febrile neutropenia in any cycle of chemotherapy

Discussion

In this population of early breast cancer patients seen in routine clinical practice at a tertiary referral centre, we identified a set of genetic factors, in addition to patient-related and chemotherapy-related factors, that predict occurrence of FN in any cycle or the first cycle

of chemotherapy. Significant predictors of a higher risk of FN in any cycle and in cycle one were: lower baseline platelet count, lower baseline haemoglobin, and carriers of the rs4148350 T-allele variant in *MRP1*, especially homozygous T-allele carriers. Patients with lower ALT and homozygous carriers of the rs246221 variant T-allele in *MRP1* and rs351855 variant T-allele in *FGFR4* had a lower risk of FN occurrence. Although the predictive ability of the models was improved by including genetic factors, the overall predictive ability remained poor. Genetic effects were stable and FN occurrence was very high in patients with specific SNP allele variants.

The observed effects of lower baseline platelet count and haemoglobin are consistent with previous reports. Baseline platelet count has been shown to differ between cancer patients with mild and severe haematological toxicity [125], and low haemoglobin has been mentioned as possible risk factor for FN [189] and survival [190]. In the model of FN occurrence in any cycle, higher baseline ALT was significantly associated with FN but not baseline bilirubin [106,191]. Both measures are indicators of liver function and since the liver detoxifies drugs like epirubicin [192], impaired liver function may be an important risk factor for FN occurrence in patients receiving chemotherapy with epirubicin. A predictive role for WBC or ANC in CIN and FN occurrence in cancer patients receiving chemotherapy has been described in other studies [104,106,130,131], but could not be confirmed in our models. Most SNPs previously associated with FN occurrence [126] and reported to be involved in anthracycline-induced cardiotoxicity [193-195] were confirmed in the multivariable analysis. The SNP rs45511401 was not included in the multivariable regression model as it was highly correlated with rs4148350, and the latter variant explained the model variability slightly better. There were no correlations between SNPs included in the final model and patient- or chemotherapy-related factors.

International guidelines [98,141,142] and the literature [106,131] report age, planned dose intensity, and planned number of chemotherapy cycles to be important risk factors for CIN and FN during chemotherapy. These risk factors could not be confirmed in our models. Patient-specific approaches to clinical management were not recorded in detail in this study and might therefore have masked the effect of age on FN occurrence. In addition, the exact cycle of FN occurrence was not available after the first cycle. Factors previously reported to protect against CIN and FN in any cycle of chemotherapy, such as dose reductions, dose delays, or growth factor use before an event occurred, could not be investigated since the details, reasons, and timing information were not available and only

15 out of 994 patients received primary prophylaxis with GCSF, mainly due to reimbursement criteria.

The apparent predictive ability, i.e., the predictive ability assessed in the 'training' dataset used to develop the models, was lower than in previously published models of CIN or FN occurrence in other cancers [105,106,130]. In these models, sensitivity and specificity at the optimal predicted probability cut-off was about 70% or higher, but in this study it remained below 70%. As commonly seen in models of FN occurrence, the NPV (≥ 90%) was much higher than the PPV because FN incidence is often around 20%; this implies an NPV of around 80% for simply assuming that FN does not occur in any patient. The areas under the ROC curves were relatively low but significantly higher than 0.5, the value indicating no predictive ability. In other words, the models allowed partial discrimination of patients at low or high risk of FN. Including genetic risk factors improved the models but absolute predictive ability remained rather low. The effects of the SNPs were stable and FN occurrence was very high in patients with specific, sometimes rare, SNP allele variants. In terms of clinical implications, genetic testing might help to identify a small proportion of patients at very high risk of FN who can be targeted with prophylactic measures. For the majority of patients, the current models do not reliably identify patients that will develop FN, but they do delineate patients who are unlikely to develop FN. This is clinically relevant since patients at low risk of FN probably do not need primary GCSF prophylaxis or nadir assessment, while the high-risk group is unpredictable and might need more extensive preventive measures or follow-up.

The performance of any model tends to be highest in the training dataset. The results obtained with bootstrap resampling supported the internal validity of the FN in any cycle and the FN in first cycle models. The predictive ability of the models has yet to be tested in an entirely independent population, where model performance is usually lower. Before risk models are put to clinical use, true external validation is essential [134,196]. Another limitation of this study is the retrospective design; no detailed information was available on patient management in clinical practice, which is known to influence the risk of FN occurrence, and the reasons and timing of dose reductions and dose delays were not available. FN occurrence was not assessed according to chemotherapy cycle beyond the first cycle. GCSF was only administered to 15 patients before an event occurred due to stringent reimbursement criteria. Hence, the impact of GCSF on FN occurrence was difficult to assess.

To the best of our knowledge, this is the first study of risk of FN in the first and any cycle of chemotherapy in patients with early breast cancer that combined a set of patient- and chemotherapy-related factors with a large set of SNPs. Further validation studies are needed to confirm our findings, which should ideally be prospectively designed, sufficiently powered, and measure all possible predictors of FN occurrence reported in the literature. Approaches to clinical management that are measurable and known to influence the risk of FN occurrence, such as dose modifications or growth factor use before an FN event occurred, should be included. Information on SNPs should be available for as many patients as possible and the frequencies of possible genotypes of one SNP should be similar. Validated genetic factors have the potential to become reliable predictors of FN occurrence. The specific SNPs that were assessed in this study are independent from clinical decision-making and therefore less likely to be confounded by clinical practice.

Conclusions

We have identified a set of chemotherapy-related, patient-related, and genetic risk factors that predict occurrence of FN in the first and any cycle of chemotherapy in a large cohort of early breast cancer patients. Genetic effects in the models improved the predictive ability, but the overall predictive ability of the models remained poor. FN occurrence was very high in patients with specific SNP allele variants. Up-front genetic testing might be helpful to identify a limited group of very high-risk patients. Further independent validation is required to develop risk models that include genetic predictors of FN occurrence and can be used to personalise care.

Competing interest

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Disclosure

AMP receives research funding from Amgen via the employing institution. HW has received lecture fees from Amgen. MS receives research funding from Amgen via the employing institution and has served on advisory boards for Amgen. RPe is on the Speaker bureau for Amgen. All the other authors declare no conflicts of interest related to this article.

Authors' contributions

AMP was responsible for analysis and data interpretation and drafted the manuscript. CV was responsible for data collection and data interpretation and helped to draft the manuscript. RPa participated in study design and data collection. ASD participated in study design and analysis and was responsible for data collection and management. RPe participated in data analysis and data interpretation. SH, PN, and DL participated in study design, data collection and data interpretation. TDS participated in data analysis and data interpretation. MS supervised data analysis and participated in data interpretation. HW was responsible for study design, participated in data collection, and the interpretation of data. All authors reviewed the manuscript and read and approved the final manuscript.

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4.3 External validation of a risk model of febrile neutropenia occurrence in patients with non-Hodgkin lymphoma

Matthias Schwenkglenks[1,5], Kate Louise Bendall[2], **Alena M. Pfeil**[1], Zsolt Szabo[3], Ruth Pettengell[4,5]

1 Institute of Pharmaceutical Medicine (ECPM), University of Basel, Basel, Switzerland

2 Biostatistics, Cascade, London, UK

3 Clinical Development, Amgen Europe GmbH, Zug, Switzerland

4 Cellular and Molecular Medicine, St. George's University of London, London, UK

5 Impact of Neutropenia in Chemotherapy – European Study Group (INC-EU)

Corresponding author:

PD. Dr. Matthias Schwenkglenks, Institute of Pharmaceutical Medicine (ECPM), University of Basel, Klingelbergstr. 61, CH-4056 Basel, Switzerland, E-mail: m.schwenkglenks@unibas.ch

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Abstract

Febrile neutropenia (FN) is a common and serious complication of chemotherapy treatment. Clinical risk models may help identify high risk FN patients but must undergo external validation before implementation in medical practice. Therefore, this study externally validated previously published clinical models of FN occurrence during chemotherapy in 240 non-Hodgkin lymphoma patients by using an independent observational dataset (N=1829). The models demonstrated predictive ability, and validation criteria for predicting any cycle FN were partially met but a larger than expected decrease in performance was noted (area under the receiver operating characteristic curve was 0.71 in the validation dataset and 0.83 in the training dataset). Age, weight, baseline white blood cell counts and planned chemotherapy parameters were confirmed to predict FN risk. Chemotherapy dose reductions, dose delays and colony-stimulating factor use were confirmed as risk modifiers during treatment. Further work is needed to improve the predictive ability of FN risk models.

Introduction

Febrile neutropenia (FN) is a serious and frequent complication in cancer patients receiving chemotherapy that may necessitate hospitalizations and intravenous antibiotic treatment, affect treatment delivery and treatment success and increase short-term mortality [101,102,179]. In addition to the clinical consequences, FN in cancer patients can cause substantial hospitalization costs [197,198]. The majority of aggressive non-Hodgkin lymphoma (NHL) patients receive chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) with or without rituximab (R) [96]. CHOP chemotherapy with a cycle length of 21 days showed FN rates of 20% or more and R-CHOP chemotherapy with a cycle length of 21 days showed rates close to 20%, in NHL patients under European routine practice conditions [97,98]. Dose dense (R-)CHOP regimens with a cycle length of 14 days have a substantially higher risk of neutropenic events and require routine administration of prophylactic colony-stimulating factor (CSF) [98,141].

Prophylactic CSF use is recommended by the European Organisation for Research and Treatment of Cancer (EORTC) and other international guidelines if the FN risk of a planned chemotherapy regimen is 20% or higher [98,141-143]. Effective targeting of CSF prophylaxis is particularly important for chemotherapy regimens with an FN risk of 10-20%, when patient risk factors must be incorporated into clinical decision making [98,141]. Antibacterial and antifungal prophylaxis have recently been recommended for neutropenic patients expected to have less than 100 neutrophils per µL for more than a week, or other risk factors for complications [144]. In patients with incurable cancer, FN risk may influence decisions on the continuation and choice of chemotherapy as well as treatment intensity. Numerous patient risk factors have been reported to increase the risk of FN including older age, low baseline blood cell counts, low serum albumin, anemia, abnormal bone marrow, increased lactate dehydrogenase (LDH), renal comorbidity, cardiovascular or hepatic disease, full dose or high-risk planned chemotherapy regimen and lack of CSF prophylaxis; and several neutropenia risk models in different cancers have been proposed [97,128,129]. Clinical risk models of FN occurrence with good predictive ability could play an important role in quantifying individual FN risk in cancer patients and targeting appropriate measures to high risk patients [127].

We previously developed risk models of FN in first and any cycle of chemotherapy using data on 240 NHL patients from the INC-EU (Impact of Neutropenia in Chemotherapy-European Study Group) Prospective Observational European Neutropenia Study [105].

The resulting models showed good test characteristics and we saw little degradation of model performance in internal 10-fold cross-validation, an approach that can be used if the available database is limited. However, before risk models can be put to clinical use they should undergo external validation in an independent dataset [134], but this occurs rarely [199]. Few neutropenia risk models have been validated using split-sample methods, i.e. dataset is randomly split-up into training set and validation set [113,130] and we are aware of only one partial validation using an independent dataset [108]. These more far-reaching validation efforts involved mixed tumor or breast cancer populations. Some of the associations found may hence be spurious or not applicable to the NHL setting.

Therefore, we aimed to assess the ability of the INC-EU models to predict the FN risk of NHL patients on external data, using the independent, observational IMPACT NHL database. If successful, the INC-EU models might help identifying high risk versus low risk patients in clinical practice.

Materials and Methods

Characteristics of the INC-EU study and model

The INC-EU Prospective Observational European Neutropenia Study was conducted in Belgium, France, Germany, Spain and the UK to assess the incidence and predictors of neutropenic events and reduced chemotherapy delivery for breast cancer and lymphoma patients undergoing chemotherapy [97,105,106]. The methodology of this study has been previously described [97]. IMPACT NHL (ClinialTrials.gov: NCT00903812) was a multi-centre, retrospective and prospective, observational study of NHL patients receiving (R-)CHOP chemotherapy. A total of 1864 patients were enrolled in 14 European countries and Australia to evaluate FN risk-assessment, FN occurrence and CSF use in routine medical practice; 1829 patients met all eligibility criteria [112,200]. Eligibility criteria were broad in both studies. Near-identity of patient characteristics was not required for the purpose of external validation as risk models should perform well in populations with partially different characteristics; otherwise their scope would be too narrow.

The characteristics of the INC-EU models have been previously published [105]. Covariates were selected based on clinical and statistical grounds. Clinically relevant risk factors significantly associated with FN in cycle 1 of chemotherapy were older age, increasing planned cyclophosphamide dose, increasing planned etoposide dose, previous chemotherapy, recent infection and low baseline albumin. The same factors, with the

exception of previous chemotherapy and low baseline albumin, were also predictive of any cycle FN occurrence. Higher weight and prophylactic CSF use were protective factors in both models; antibiotic prophylaxis had no significant effect and was therefore excluded [105]. The following additional factors were statistically significant predictors of risk of any cycle FN: low baseline absolute neutrophil count (ANC) or white blood cell count (WBC), high baseline alkaline phosphatase, cardiovascular comorbidity and increasing planned cytarabine dose. Chemotherapy dose reductions and dose delays before an FN event occurred decreased the risk of FN. The INC-EU models demonstrated good apparent predictive ability; apparent in that performance was assessed directly in the dataset used to develop the models. Predictive ability was only slightly reduced under 10-fold cross validation conditions.

Feasibility of comparing the INC-EU and IMPACT NHL studies was assessed. In the INC-EU study, FN was defined as a temperature of ≥38.0˚C in conjunction with an ANC $<0.5\times10^9$/L or WBC $<1.0\times10^9$/L [105]. IMPACT NHL used a slightly broader definition as a neutrophil count $\leq1.0\times10^9$/L was sufficient if predicted to fall below $<0.5\times10^9$/L [112,200]. The exact time points of FN events were not directly available from the IMPACT NHL data. CSF use in the relevant chemotherapy cycles was therefore assumed to precede the FN event if it started on cycle days 1-7. Definitions of patient demographics or characteristics (used as covariates in the models) were reasonably similar or could be aligned by applying INC-EU definitions to IMPACT NHL.

Covariates representing high baseline alkaline phosphatase (used in the INC-EU model of FN risk in any cycle) and recent infection were not available from the IMPACT NHL dataset. A sensitivity analysis using the INC-EU dataset investigated the effect of omitting these variables and showed that they contributed little to the risk models' overall predictive ability (area under the receiver operating characteristic (ROC) curve and test characteristics remained stable when they were excluded, as did the regression coefficients estimated for the other predictor variables). However, the most important predictors of FN such as age, previous chemotherapy, planned chemotherapy dose, CSF use, and baseline neutrophil counts were available for both study populations or could be derived from the IMPACT NHL dataset.

External validation of the INC-EU models

The external validation followed a double approach. First, we assessed the performance of the INC-EU risk models when applied to the IMPACT NHL database. The logistic

regression coefficients constituting the INC-EU models were combined with covariate values from the IMPACT NHL population. Linear predictors for risk of FN in cycle 1 and in any cycle were calculated for 1818 and 1675 IMPACT NHL patients, respectively, with no missing values for the relevant variables, and converted to predicted probabilities. These predicted probabilities for FN were compared with the patients' actual FN experience (occurrence yes versus no) in the first or any cycle of chemotherapy. Predictive ability was assessed by calculating the area under the ROC curve and test characteristics such as sensitivity, specificity, positive predictive value (PPV, proportion of patients classified as high risk by the model who actually have an event) and negative predictive value (NPV, proportion of patients classified as low risk by the model who actually have no event). Test characteristics were calculated at the optimal cut-off (i.e. the cut-off where sensitivity equals specificity) observed for both the INC-EU and IMPACT NHL datasets, and at a cut-off of 0.5. We pre-specified that the risk models would be regarded as formally successfully validated if the sensitivity and specificity were no less than the observed values for the INC-EU dataset minus 10%, and if the area under the ROC curve was greater than 0.75 (compared to 0.86 and 0.83 for the INC-EU dataset), based on the recommendations of published validation reports [201-203].

Second, to generate supplementary information, logistic regression coefficients were re-estimated using the IMPACT NHL database, i.e. the effects deemed significant in the INC-EU models were re-estimated from the IMPACT NHL data and compared with the original regression coefficient estimates.

Results

The INC-EU risk model was based on 240 NHL patients and the IMPACT NHL full analysis set used for validation included 1829 NHL patients. As presented in Table 4.3-1, the INC-EU and IMPACT NHL populations showed similar treatment and patient characteristics. FN occurred in cycle 1 in 21 (9%) and in 127 (7%) patients in the INC-EU and IMPACT NHL studies, respectively, and was reported in any cycle FN in 53 (22%) and in 331 (18%) patients, respectively.

Ability of the INC-EU models to predict FN in the IMPACT NHL database

The INC-EU risk models predicted the occurrence of FN in cycle 1 and in any cycle in IMPACT NHL patients (Table 4.3-2).

Table 4.3-1: Baseline demographics and disposition of study population

	IMPACT NHL (N = 1829)	**INC-EU[*] (N = 240)**
Tumor Type		
Diffuse large B-cell lymphoma (DLBCL)	1136 (62%)	154 (64%)
Follicular lymphoma (FL)	345 (19%)	35 (15%)
Other	348 (19%)	51 (21%)
Regimen received		
CHOP-14	536 (29%)	41 (17%)
CHOP-21	1293 (71%)	178 (74%)
Other	0	21 (9%)
Planned to receive Rituximab	1698 (93%)	196 (82%)
Number of planned cycles		
≤4 cycles	239 (9%)	51 (21%)
5 - 6 cycles	1027 (56%)	105 (44%)
>6 cycles	563 (31%)	84 (35%)
Age - mean (SD)	60.2 (13.9)	63.2 (12.9)
Age≥65 years	805 (44%)	130 (54%)
Female	803 (44%)	105 (44%)
Ann Arbor Stage 3-4	1142 (62%)	133/237 (56%)
International Prognostic Index (IPI) intermediate/high	849/1484 (57%)	162/237 (68%)
FLIPI intermediate/High	251/345 (73%)	not available
Weight (kg) – mean (SD)	73.9 (15.5)	75 (16)
Prior chemotherapy (chemo- and/or radiotherapy)	143 (8%)	25 (10%)
Low baseline albumin <35 g/dl	254 (14%)	54/188 (29%)
Missing baseline albumin	628 (34%)	52/240 (22%)
Low baseline absolute neutrophil count<3 or WBC<5	377 (21%)	52/237 (22%)
Cardiovascular comorbidities	398 (22%)	65 (27%)

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; FLIPI, Follicular Lymphoma International Prognostic Index; INC-EU, Impact of Neutropenia in Chemotherapy-European Study Group; NHL, non-Hodgkin lymphoma; WBC, white blood cell count

[*] Based on INC-EU dataset and as reported in Pettengell*, et al.* 2009 [105].

Table 4.3-2: Risk model performance in the INC-EU (training) and IMPACT NHL (external validation) dataset

	FN in first cycle			**FN in any cycle**		
	INC-EU (training dataset)*	IMPACT NHL (validation dataset)	IMPACT NHL (validation dataset)	INC-EU (training dataset)*	IMPACT NHL (validation dataset)	IMPACT NHL (validation dataset)
Choice of cut-off	Optimal for INC-EU model	Optimal for INC-EU model	Optimal for IMPACT NHL model	Optimal for INC-EU model	Optimal for INC-EU model	Optimal for IMPACT NHL model
Cut-off value	0.116	0.116	0.014	0.232	0.232	0.089
Correct predictions (%)	192 (80)	1583 (87)	1074 (59)	180 (76)	1306 (78)	1113 (66)
Area under the ROC curve (95% CI)	0.86 (0.79-0.94)	0.64 (0.59-0.69)		0.83 (0.76-0.90)	0.71 (0.68-0.75)	
Sensitivity (%)	81	14	59	76	42	66#
Specificity (%)	80	93	59	76	86	67#
Negative predictive value (%)	98	93	95	92	87	90
Positive predictive value (%)	28	13	10	48	40	30

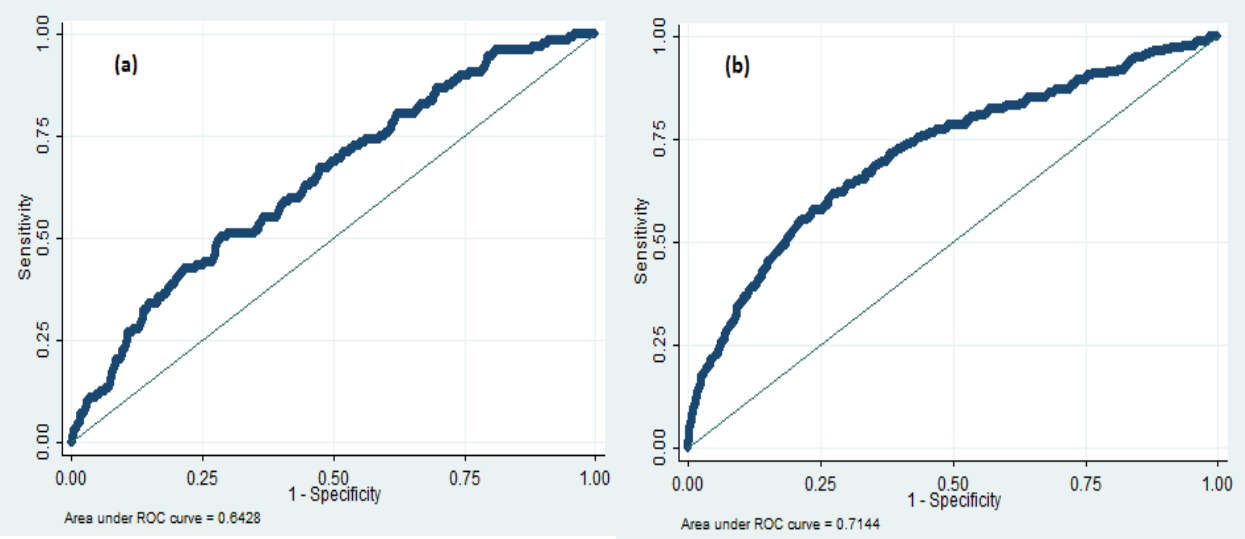
CI, confidence interval; FN, febrile neutropenia; INC-EU, Impact of Neutropenia in Chemotherapy-European Study Group; NHL, non-Hodgkin lymphoma; ROC, receiver operating characteristic

* As published previously (Pettengell, et al. 2009 [105]).

Formal criterion for successful validation met.

For FN in cycle 1, the area under the ROC curve was 0.64 (95% confidence interval [CI] 0.59-0.69) (Figure 4.3-1) and 0.71 (CI 0.68-0.75) for FN in any cycle (Figure 4.3-1). In both cases, the lower confidence limit for the area under the ROC curve was significantly higher than 0.5, i.e. above the value that would indicate no predictive ability. Overall, 1074 out of 1818 patients (59%) were classified correctly in the first cycle; 75 (4%) positively predicted patients experienced FN and 999 (55%) negatively predicted patients did not experience FN. Correct predictions in any cycle FN were higher; overall, 1113 patients out of 1675 (66%) were correctly classified; 201 (12%) positively predicted patients experienced FN and 912 (54%) negatively predicted patients did not experience FN. These results were calculated using the optimal cut-off observed for the IMPACT NHL dataset (0.014 for FN in cycle 1 and 0.089 in any cycle). When the other cut-offs were used, test characteristics shifted towards lower sensitivity and higher specificity. When using a cut-off of 0.5, sensitivity was very low in the first cycle model (2%) and low in the any cycle model (18%), specificity was very high in both models (99% and 97%) and the NPV was 93% in the first cycle model and 85% in the any cycle model.

Figure 4.3-1: ROC curves for FN in cycle 1 and FN in any cycle



ROC, receiver operating characteristic

Bisecting line indicates a discrimination that is no better than chance (ROC=0.5), no predictive ability

Re-estimation of model parameters from the IMPACT NHL database

When the model parameters of the first cycle INC-EU [105] FN model were re-estimated using the IMPACT NHL dataset, all effects maintained their original direction (with the exception of missing baseline albumin, a category that was introduced not to lose too many observations, as baseline albumin was not recorded for all patients), but some effect sizes

were substantially reduced (e.g. age, previous chemotherapy) and the majority of effects were not statistically significant (Table 4.3-3). The re-estimated model obtained an apparent area under the ROC curve of 0.67 (CI 0.62-0.72) compared to 0.86 (CI 0.79-0-94) for the original INC-EU model of cycle 1 FN [105].

In the any cycle model, all effects maintained their original direction and in this case effects remained statistically significant (with the exception of previous chemotherapy and cardiovascular comorbidity). Again, most effect sizes were reduced (Table 4.3-3). Planned cycle length, distinguishing dose-dense ([R-]CHOP-14) from standard chemotherapy delivery ([R-]CHOP-21), was not statistically significant in the INC-EU any cycle model but was statistically significant in the re-estimated model. The re-estimated model obtained an apparent area under the ROC curve of 0.74 (CI 0.70-0.77) compared to 0.83 (CI 0.76-0.90) for the corresponding INC-EU model of FN in any cycle [105].

Ad Hoc analysis of baseline albumin

In the original INC-EU models [105], the effect of low baseline albumin was significant in the FN in cycle 1 model but failed to reach significance in the FN in any cycle model. In an unplanned *ad hoc* analysis it was assessed whether low baseline albumin would be predictive of FN in any cycle in the IMPACT NHL data. The addition of low baseline albumin as a covariate to the IMPACT NHL models confirmed an effect of low baseline albumin on the incidence of FN in any cycle. The resulting coefficient (OR = 1.80, CI 1.3-2.5) was highly significant. In light of this, the impact of including low baseline albumin in the published INC-EU model (FN in any cycle) on the ability to predict FN in the IMPACT NHL dataset was investigated. Little impact of adding low baseline albumin was seen and consequently the predictive ability of the modified algorithm was not substantially increased in comparison to the algorithm resulting from the published INC-EU model.

Table 4.3-3: INC-EU model of FN risk in first and any cycle: comparison of original model parameters and re-estimated model parameters based on IMPACT NHL dataset

	FN in first cycle				**FN in any cycle**			
	Original model parameters*		Re-estimated model parameters#		Original model parameters*		Re-estimated model parameters#	
Covariates	Odds ratio (95% CI)	p value§	Odds ratio (95% CI)	p value§	Odds ratio (95% CI)	p value§	Odds ratio (95% CI)	p value§
Age┼	2.20 (1.21-4.01)	0.01	1.16 (0.96-1.41)	0.13	1.79 (1.16-2.78)	0.01	1.43 (1.24-1.65)	< 0.01
Weight**	0.62 (0.43-0.89)	0.01	0.89 (0.78-1.03)	0.22	0.62 (0.44-0.88)	0.01	0.90 (0.84-0.98)	0.01
Cardiovascular comorbidity	-		-		2.56 (1.04-6.29)	0.04	1.16 (0.82-1.65)	0.39
Low baseline ANC or WBC##	-		-		4.18 (1.82-9.60)	< 0.01	1.92 (1.38-2.67)	< 0.01
Previous chemotherapy	6.39 (1.72-23.68)	<0.01	1.46 (0.79-2.70)	0.22	1.76 (0.49-6.36)	0.39	1.02 (0.61-1.70)	0.94
Planned cyclophosphamide dose§§	1.16 (1.02-1.32)	0.02	1.12 (0.98-1.27)	0.10	1.33 (1.16-1.52)	< 0.01	1.14 (1.04-1.26)	0.01
Planned cytarabine dose§§	1.06 (0.98-1.16)	0.15	Not administered in IMPACT NHL		1.09 (1.05-1.13)	<0.01	Not administered in IMPACT NHL	
Planned etoposide dose§§	1.59 (1.20-2.11)	<0.01			1.27 (1.03-1.57)	0.02		
Dose dense regimen (cycle length 2 weeks)	-		-		1.84 (0.71-4.78)	0.21	2.07 (1.45-2.95)	< 0.01
CSF use before an event occurred┼┼	0.18 (0.03-0.94)	0.04	0.48 (0.30-0.77)	0.00	0.21 (0.10-0.44)	< 0.01	0.45 (0.32-0.64)	< 0.01
Dose reduction before an event occurred┼┼	-		-		0.24 (0.09-0.63)	< 0.01	0.32 (0.21-0.48)	< 0.01

Dose delay before an event occurred[††]	-		-		0.17 (0.07-0.40)	< 0.01	0.39 (0.28-0.55)	< 0.01
Baseline albumin low[***]	4.76 (1.35-16.71)	0.02	3.15 (1.98-5.01)	0.00	-		-	
Baseline albumin missing[***]	0.52 (0.09-2.99)	0.46	1.54 (0.99-2.39)	0.06	-		-	
Baseline alkaline phosphatase high[###]	-		Not recorded in IMPACT NHL		9.07 (1.41-58.50)	0.02	Not recorded in IMPACT NHL	
Baseline alkaline phosphatase missing[###]	-				4.75 (0.73-30.84)	0.10		
Recent infection[§§§]	3.07 (0.99-9.52)	0.05			3.32 (1.03-10.71)	0.04		

ANC, absolute neutrophil count; CI, confidence interval; CSF, colony-stimulating factor; NHL, non-Hodgkin lymphoma; WBC, white blood cell count.

[*] Based on INC-EU dataset and as reported in Pettengell*, et al.* 2009 [105]; N = 237 usable observations.

[#] Based on IMPACT NHL dataset; N = 1818 usable observations for cycle 1 models and N = 1675 usable observations for any cycle model.

[§] Based on general estimating equations-based robust standard error estimates allowing for clustering by study site.

[†] Per additional 10 years of age.

[**] Per additional 10 kg body weight.

[##] Baseline ANC < 3.0 x 10^9/l or WBC < 5.0 x 10^9/l.

[§§] Per additional mg/m^2 body surface area/week; per additional 50 mg/m^2.

[††] Myelopoietic growth factor use; chemotherapy dose reduction; chemotherapy dose delay before a FN event occurred.

[***] Baseline albumin <35 g/dl, missing category introduced to avoid loss of observations

[###] Baseline alkaline phosphatase > 250 iU/l, missing category introduced to avoid loss of observations

[§§§] During 60 d prior to chemotherapy or ongoing infectious comorbidity

Discussion

Models that identify and quantify individual FN risk factors can aid clinical practice and facilitate adherence to guideline recommendations. Our risk models for NHL patients demonstrated some ability to predict FN occurrence in cycle 1 and in any cycle of chemotherapy, in the entirely independent IMPACT NHL database. The observed high NPV (≥90%) relative to PPV is common to many risk models, here reflecting the ratio of patients without *versus* with FN (e.g., an overall FN incidence in the IMPACT study of 18% translates to a NPV of 82% in the absence of modelled risk assessment). However, formal pre-defined criteria for successful validation were not met for the cycle 1 model. The any cycle model, met criteria for sensitivity and specificity but not those for area under the ROC curve. The overall decrease in performance in the external validation dataset compared to the training dataset was larger than expected. Application to individual patients in routine practice would probably not achieve a sufficiently precise prediction of actual FN risk.

A number of the covariates used in the original INC-EU models were confirmed to be important predictors of FN and are likely to be important elements of future (more refined) prediction models. These covariates included age, weight, low baseline ANC or WBC, planned chemotherapy cycle length and planned cyclophosphamide dose (where applicable). Chemotherapy dose reductions and delays, and CSF use were confirmed as important modifiers of FN risk during the course of chemotherapy. They can decrease the risk of FN in subsequent cycles with impact on the overall risk, but their use is often triggered by the observed or anticipated FN risk in early cycles. Effects of previous chemotherapy (incidence of previous chemotherapy was <10% in both the INC-EU and the IMPACT NHL data) and cardiovascular comorbidity could not be confirmed. The capture of cardiovascular comorbidity data in IMPACT NHL was not well standardized; only system organ class comorbidity data were collected without including further details on specific disease states. Consequently, the potential for correctly assessing the impact of these covariates may have been limited. Low albumin is representative of poor nutritional condition. An unplanned extension of our external validation analyses suggested that low baseline albumin may be an influential contributor to the risk of FN. Although its impact on the predictive ability of the models was limited, clinical decision making should possibly take this element into account when assessing patient risk factors. The true effect of low baseline albumin warrants further investigation.

The predictive ability of the original INC-EU models of FN occurrence, when applied to

independent lymphoma datasets, is unlikely to be sufficient for effective prediction. As validated risk models of FN occurrence in NHL patients and other cancer patients are rare, there is a lack of materials our results can be compared with. The apparent predictive ability of our models resembled that of a model recently published by Lyman, *et al.,* in which risk of severe and febrile neutropenia in cycle 1 was assessed in patients with solid tumors and malignant lymphomas [130]. These authors reported an apparent NPV of 96% and PPV of 34%. Within-study 2:1 random split-sample validation resulted in a slightly increased PPV (36%) but lower NPV (93%). The fact that our validation study used data on NHL patients enrolled into an entirely different study may explain the much larger performance change seen in our case [130,204]. A risk model developed by López-Pousa, *et al.* [131] for first cycle chemotherapy-induced neutropenia in 1194 patients with solid tumors had slightly lower apparent predictive ability (PPV of 17% and NPV of 94%) than our model. To our knowledge, this model has not been validated externally. The observed differences in model performance under different conditions and especially the difference in performance change that may result from the use of random split-sample validation *versus* true external validation confirms the importance of the latter approach before risk models are put to clinical use.

Using prospectively collected data (in the INC-EU study and part of the IMPACT NHL study) to develop and validate prognostic models may add strength as such data may be more reliable than retrospectively collected data. Possible limitations of our work include the small training dataset sample size (N = 240), the mixture of first line and relapsed chemotherapy patients with different treatment histories (although ≤10% in both datasets had previous chemotherapy) and treatment intensity, slight differences in some definitions between the IMPACT NHL study and the INC-EU study and unavailability of two covariates of limited relevance in IMPACT NHL. However, we consider only the limited size of the training set has possibly contributed to the decrease in performance. Another possible explanation could be the lack of accounting for other, unknown prognostic covariates such as biological or genetic risk factors [205]. In addition, correct representation of the neutropenic potential of combination chemotherapy regimens remains a challenge. The dosage of the individual components is often correlated (e.g. patients with higher body surface area get a proportionally higher dose of each substance). This makes it difficult to correctly estimate the risk associated with each individual agent. Moreover, agents are used in different combinations (although not so much here where CHOP was predominantly used); to our knowledge, their interaction has not been systematically

studied in routine practice populations, with a focus on implications for FN risk. An additional issue that arises when risk models are put to clinical use is choice of cut-off value. Related decision making cannot be solely based on statistical criteria. For example, the main clinical focus may be on high PPV (i.e. selection of a patient group that certainly needs prophylaxis) or rather on high NPV (i.e. selection of a patient group where prophylaxis can be safely omitted).

The current study indicates that the efficient identification of patients at high risk of FN continues to face serious challenges. Additional strategies are required for future research before FN risk models can be incorporated into routine clinical practice. As a first step, systematic literature reviews (documenting all currently proposed FN risk factors with supporting evidence), hypothesis-driven reanalyses of existing data, and, where required, well-defined primary data collections should be used to define the most promising elements. These elements should be consistent with our knowledge of the pathophysiology of chemotherapy-induced FN, or at least repeatable in independent clinical studies. Additional criteria would include high predictive ability and easy applicability in routine practice situations. The resulting set of candidate predictors would inform thorough, sufficiently powered, prospective cohort studies generating comprehensive datasets for risk model generation and validation. Under ideal circumstances, additional clinical and health economic evidence for the resulting prediction models would be obtained from randomized controlled studies comparing standard medical strategies with medical strategies dependent on predicted individual risk [206]. The primary endpoint of FN occurrence should be complemented with secondary endpoints including FN-related mortality and hospitalisation for FN. In the real-world, feasibility aspects and timeliness may require more limited approaches.

It should additionally be noted that even validated risk models would only affect patients' health outcomes if they influenced physicians' treatment decisions [207]. Salar, *et al.* [112] showed that although in the IMPACT NHL population about 60% of the patients were assessed as being at high risk of FN, less than half received primary prophylactic CSF as per guideline recommendations [98,141,143,142], indicating that there is an additional risk assessment done by physicians. As some guideline recommendations remain rather vague, risk models could be expected to provide clearer guidance for distinguishing high risk from low risk patients. Even models with limited predictive ability may make physicians more aware of relevant risk criteria. Comparative studies evaluating how the availability of

risk models influences physician behaviour and health outcomes [207] might help to promote evidence-driven risk prediction and optimize clinical and supportive care together with physicians' decision making on chemotherapy use, patient surveillance and the need for prophylaxis.

The limited performance of our risk models highlights the importance of careful clinical decision making until validated models are available with adequate predictive ability. Therefore, there is a clear need for further studies and continuing validation of proposed risk predictors and tools.

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M.S., K.L.B. and R.P. designed the research study. M.S. and K.L.B. performed the modelling analysis. All authors analysed and interpreted the data and wrote the paper; M.S. and A.M.P. prepared the first draft. The authors are fully responsible for content and editorial discussion for this manuscript.

Disclosure and competing interests

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M.S. receives research funding from Amgen via employment institution and has served on advisory boards for Amgen. K.L.B. is a consultant and contract worker at Amgen Ltd. Z.S. is an employee of Amgen (Europe) GmbH. A.M.P. receives research funding from Amgen via employment institution. R.P. is a consultant in receipt of honoraria from Chugai, Bayer, Roche, and Amgen and has served on advisory boards for Bayer, Amgen and Roche.

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4.4 Efficacy, effectiveness and safety of long-acting granulocyte colony-stimulating factors for prophylaxis of chemotherapy-induced neutropenia in patients with cancer: a systematic review

Alena M. Pfeil[1], Kim Allcott[2], Ruth Pettengell[3], Gunter von Minckwitz[4], Matthias Schwenkglenks[1*], Zsolt Szabo[5*]

* Joint last authorship

1 Institute of Pharmaceutical Medicine, University of Basel, Basel, Switzerland

2 Oxford PharmaGenesis™ Ltd, Oxford, UK

3 Cellular and Molecular Medicine, St. George's University of London, London, UK

4 German Breast Group, Neu-Isenburg and University of Frankfurt, Frankfurt, Germany

5 Clinical Development, Amgen Europe GmbH, Zug, Switzerland

Corresponding author:

Alena M. Pfeil, Institute of Pharmaceutical Medicine (ECPM), University of Basel, Klingelbergstr. 61, CH-4056 Basel, Switzerland, E-mail: alena.pfeil@unibas.ch

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Abstract

Purpose: Pegfilgrastim was introduced over a decade ago. Other long-acting granulocyte colony-stimulating factors (G-CSFs) have recently been developed. We systematically reviewed the efficacy, effectiveness and safety of neutropenia prophylaxis with long-acting G-CSFs in cancer patients receiving chemotherapy.

Methods: We performed a systematic literature search of the MEDLINE, EMBASE and Cochrane Library databases, and abstracts from key congresses. Studies of long-acting G-CSFs for prophylaxis of chemotherapy-induced neutropenia (CIN) and febrile neutropenia (FN) were identified by two independent reviewers. Abstracts and full texts were assessed for final inclusion; risk of bias was evaluated using the Cochrane's tool. Effectiveness and safety results were extracted according to study type and G-CSF used.

Results: Of the 839 articles identified, 41 articles representing different studies met the eligibility criteria. In five randomised controlled trials, 11 clinical trials and 17 observational studies across several tumor types and chemotherapy regimens, pegfilgrastim was used alone or compared with daily G-CSF, no G-CSF, no upfront pegfilgrastim, or placebo. Studies generally reported lower incidence of CIN (4/7 studies), FN (11/14 studies), hospitalisations (9/13 studies) antibiotic use (6/7 studies), and adverse events (2/5 studies) with pegfilgrastim than filgrastim, no upfront pegfilgrastim or no G-CSF. Eight studies evaluated other long-acting G-CSFs; most (5/8) were compared to pegfilgrastim and involved patients with breast cancer receiving docetaxel-based therapy. Efficacy and safety profiles of balugrastim and lipegfilgrastim were comparable to pegfilgrastim in phase 3 studies. Efficacy and safety of other long-acting G-CSFs were mixed.

Conclusions: Pegfilgrastim reduced the incidence of FN and CIN compared with no prophylaxis. Most studies showed better efficacy and effectiveness for pegfilgrastim than filgrastim. Efficacy and safety profiles of lipegfilgrastim and balugrastim were similar to pegfilgrastim.

Introduction

In patients with cancer receiving cytotoxic chemotherapy, chemotherapy-induced neutropenia (CIN) and febrile neutropenia (FN) are frequent complications. CIN is graded according to severity of the reduction of the absolute neutrophil count (ANC) and FN is commonly defined as ANC $<0.5 \times 10^9$/L with an oral temperature $\geq$38°C for more than 1 hour [142]. Patients experiencing neutropenic events are more susceptible to subsequent infections [99]. As a consequence of FN, patients often require hospitalisation and antibiotic treatment and frequently have their chemotherapy dose reduced or delayed [103,208]. Modifications to chemotherapy may decrease its effectiveness, thereby potentially compromising treatment outcomes [103].

Granulocyte colony-stimulating factors (G-CSFs) stimulate the production and maturation of neutrophils during chemotherapy and reduce the incidence and duration of CIN and incidence of FN [139,209]. Prophylactic G-CSF use from the first cycle of chemotherapy is recommended by the European Organisation for Research and Treatment of Cancer (EORTC) [98] and other international guidelines [141-143] if the planned chemotherapy regimen is associated with an FN risk of 20% or more. For chemotherapy regimens with an intermediate FN risk of 10–20% guidelines recommend that patient-related and disease-related factors should also be considered when deciding whether or not to give G-CSF support.

Daily G-CSFs are primarily cleared through the kidneys and require dosing until recovery of the neutrophil count. Long-acting G-CSFs are primarily cleared by neutrophils and have significantly reduced renal clearance compared with daily G-CSFs. They therefore require only a single dose per chemotherapy cycle. Pegfilgrastim (Neulasta®, Amgen Inc., CA, USA), consisting of the human recombinant G-CSF filgrastim pegylated at the N-terminus with a 20 kilodalton polyethylene glycol molecule, is administered subcutaneously as a single 6 mg dose [210]. It was approved in both the USA and Europe in 2002. Lipegfilgrastim (Lonquex®, Teva Pharma B. V.), a long-acting filgrastim molecule that is pegylated at a different site from pegfilgrastim, was approved in Europe in 2013 [211]. Other long-acting G-CSFs, such as balugrastim, are in clinical development [212].

The emergence of these recently-developed long-acting G-CSFs necessitates a re-evaluation of the evidence. Direct comparative data are limited, and there are no systematic reviews of long-acting G-CSFs that include data from both observational

studies and randomised controlled trials (RCTs). Therefore, we conducted a systematic review to capture the available data on the efficacy, safety and effectiveness of long-acting G-CSFs for prophylaxis of CIN and FN in adult patients with cancer.

Methods

Study design

The systematic review was performed according to a pre-specified protocol that was agreed by all authors. We searched the following electronic databases: MEDLINE In-Process & Other Non-Indexed Citations and OVID MEDLINE 1948-present, EMBASE 1980-present and the Cochrane Library. A search of abstract books was also conducted from the annual meetings of the American Society of Clinical Oncology, the American Society of Hematology, the European Hematology Association, the European Society for Medical Oncology, the European Multidisciplinary Cancer Congress, the International Society for Pharmacoeconomics and Outcomes Research and the Multinational Association of Supportive Care in Cancer. Complete search strings are listed in Appendix. The electronic database searches included articles published up to April 2013 and were restricted to English-language studies. Conference abstracts were limited to those published between January 2009 and April 2013. This report follows the PRISMA statement for reporting systematic reviews and meta-analyses [150].

Study Selection

Initially, two independent reviewers screened the titles and abstracts of the search results for studies of human adult haematology or oncology patients who were receiving long-acting-G-CSF primary prophylaxis to reduce the risk of CIN during chemotherapy. Studies in which patients received bone marrow transplantation were excluded. Clinical trials and observational studies were included. Editorials, letters, case reports, guidelines, health technology assessment reports, economic evaluations, narrative reviews and research protocols were excluded. Papers were excluded if they did not report neutropenia-related outcomes. Full texts of the remaining articles were then assessed by the reviewers for final inclusion. Additional exclusion criteria were applied at this second stage: studies comparing pegfilgrastim with a daily G-CSF, placebo or no prophylaxis were excluded if fewer than 50 patients received pegfilgrastim; studies with pegfilgrastim alone (which therefore allowed no comparisons) were excluded if fewer than 100 patients received pegfilgrastim. Studies in which pegfilgrastim was used outside of its approved indication were excluded. These additional exclusion criteria were not applied to studies involving

new long-acting G-CSFs because we expected to find far fewer papers on these and wanted to ensure that all available data on these other agents were captured. Papers or abstracts reporting results from the same study were indicated as such. If a study included in the form of a congress abstract was published as a peer-reviewed paper after our literature search, we included the paper in place of the congress abstract.

Data extraction

The data collection comprised study and patient characteristics, efficacy (effect of a treatment under controlled, clinical trial conditions), effectiveness (effect of a treatment under uncontrolled, real-world conditions) and safety. Detailed definitions of outcome measures are listed in Appendix. Studies were classified according to their design: 'RCTs' where patients were randomised to G-CSFs; clinical trials in which patients were not randomly assigned to neutropenia prophylaxis or no treatment were termed 'clinical trials'; and studies of routine clinical practice were termed 'observational studies'. Evidence found in the literature was extracted as presented by the original authors of the study.

Risk of bias assessment

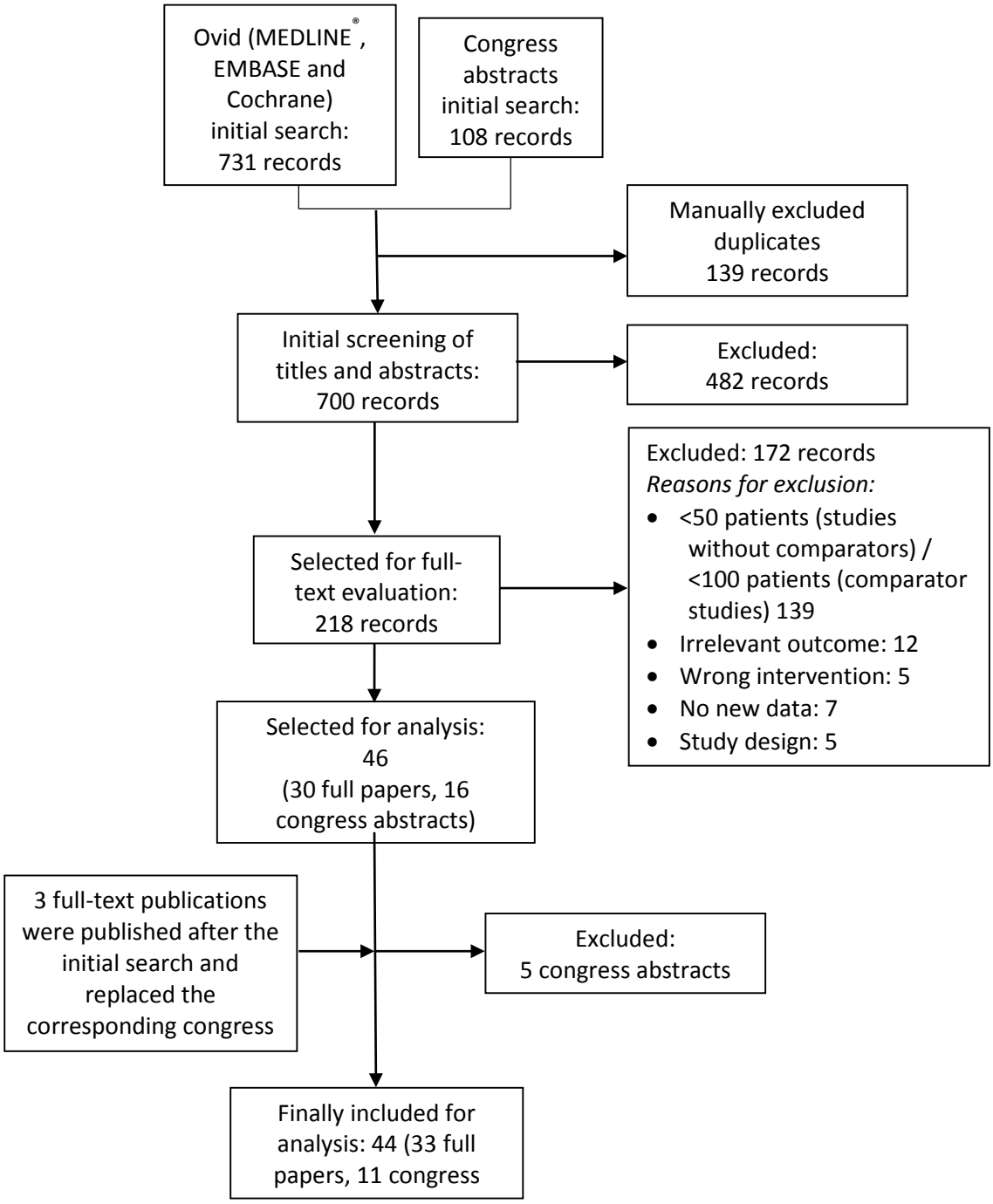
Two independent reviewers assessed risk of bias; disagreements were resolved within the reviewer team by consensus. RCTs were assessed using the Cochrane Collaboration's assessment tool [213]. Non-randomised studies were assessed using the Methods Guide for Comparative Effectiveness Reviews of the US Agency for Healthcare Research and Quality [214]. Six domains of bias (selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias) were assessed. Based on the reviewers' judgments, every article was rated as having a 'low', 'high' or 'unclear' risk of bias. Risk of bias was not assessed for conference abstracts.

Results

Eligible trials and study characteristics

Our search identified 731 full publications and 108 congress abstracts (Figure 4.4-1). After removing duplicates, 700 items were left, of which 482 were excluded on the basis of title and abstract screening, leaving 218 articles (Supplementary material of the journal, ESM 3). Three relevant articles were published after completion of the search: Bondarenko et al. (2013) [215], Almenar-Cubells et al. (2013) [216] and Volovat et al. (2013) [217]; these were included to replace congress abstracts identified by the initial search that described the same studies [218-220].

Figure 4.4-1: PRISMA flow diagram



Finally, 33 publications and 11 congress abstracts representing 41 studies were analysed. Key characteristics of the included studies are presented in Table 4.4-1.

Figure 4.4-2 illustrates the number of patients exposed to each of the included substances or treatment strategies, the G-CSF interventions used, and the study design. The studies included 13 that looked at pegfilgrastim alone, 15 studies in which pegfilgrastim was compared with a daily G-CSF, three studies in which pegfilgrastim was compared with placebo and two studies in which pegfilgrastim primary prophylaxis was compared with no pegfilgrastim primary prophylaxis. We found eight studies that compared other long-acting G-CSFs with daily G-CSFs, pegfilgrastim, or placebo. The number of patients who received a long-acting G-CSF was 50,089 (pegfilgrastim = 49,207; lipegfilgrastim = 505; balugrastim = 281; Maxy-G34 = 27; Ro 25-8315 = 28; BCD-017 = 41).

Pegfilgrastim studies included patients with breast, lung, colorectal or gastro-esophageal cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, acute myeloid leukaemia and various other solid tumours. These studies included patients taking 12 standard chemotherapy regimens and numerous non-standard regimens. All studies of newer long-acting G-CSFs except one (which looked at lipegfilgrastim in non-small-cell lung cancer [221]) were conducted in patients with breast cancer, most of whom were receiving docetaxel and doxorubicin.

Table 4.4-1: Overview of study and patient characteristics of included studies

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
RCTs							
Bondarenko et al. 2013 [215]	RCT	Doxorubicin/ docetaxel	Lipegfilgrastim (6 mg) *n* = 101 Pegfilgrastim (6 mg) *n* = 101	Duration of SN in cycle 1	Breast cancer	Lipegfilgrastim 49.9 ± 10.1 Pegfilgrastim 51.1 ± 9.4	4 cycles
Buchner et al. 2011 [222] congress abstract	RCT	Doxorubicin/ docetaxel	Lipegfilgrastim (3 mg; 4.5 mg; 6 mg) *n* = 53; *n* = 51; *n* = 50 Pegfilgrastim (6 mg) *n* = 54	Duration of SN in cycle 1	Primary breast cancer	–	4 cycles
Gladkov et al. 2012 [223] congress abstract	RCT	Doxorubicin/ docetaxel	Balugrastim (40 mg; 50 mg) or Pegfilgrastim (6 mg) *n* = 256	Duration of SN in cycle 1	Breast cancer	–	–
Green et al. 2003 [224]	RCT	Doxorubicin/ docetaxel	Pegfilgrastim (6 mg) *n* = 77 Filgrastim (5 µg/kg/day) *n* = 75	Duration of grade 4 neutropenia in cycle 1	High-risk breast cancer	Pegfilgrastim 52.1 (31–75) Filgrastim 52.8 (30–74)	4 cycles
Holmes et al. 2002 [225]	RCT	Doxorubicin/ docetaxel	Pegfilgrastim (100 µg/kg) *n* = 150 Filgrastim (5 µg/kg/day) *n* = 151	Duration of grade 4 neutropenia in cycle 1	Breast cancer	Pegfilgrastim 50.9 ± 11.7 Filgrastim 51.9 ± 11.1	4 cycles
Salafet et al. 2013 [226] congress abstract	RCT	Doxorubicin/ docetaxel	BCD-017 (3 mg; 6 mg) *n* = 21; *n* = 20 Filgrastim (5 mg/kg/day) *n* = 19	Incidence of SN	Breast cancer	–	–

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
Viens et al. 2002 [227]	RCT	Doxorubicin, cyclo-phosphamide	Ro 25-8315 (20 µg/kg; 60 µg/kg; 100 µg/kg) *n* = 9; *n* = 9; *n* = 10 Filgrastim (5 µg/kg/day) *n* = 8	Peak of circulating CD34+ cells and duration of CIN	Advanced breast cancer	50* (39-57) 54* (37-59) 54* (37–61) Filgrastim 52* (32–56)	–
Vogel et al. 2005 [228][#]	RCT	Docetaxel	Pegfilgrastim (6 mg) *n* = 463 Placebo *n* = 465	Incidence of FN	Breast cancer	Pegfilgrastim 51.9* (21–88) Placebo 52.1* (24–76)	4 cycles
Volovat et al. 2013 [217][§]	RCT	Doxorubicin/docetaxel	Balugrastim (40 mg) *n* = 153 Pegfilgrastim (6 mg) *n* = 151	Duration of SN in cycle 1	Breast cancer	Balugrastim 51.5 ± 10.3 Pegfilgrastim 50.8 ± 9.7	4 cycles
Gladkov et al. 2012 [221] congress abstract	RCT	Cisplatin/ etoposide	Lipegfilgrastim (6 mg) *n* = 250 Placebo *n* = 125	Incidence of FN during cycle 1	NSCLC	–	–
Decaestecker and Pinter et al. 2013 [229,230] Cong. abstracts	RCT	FOLFOX/FOLFIRI plus bevacizumab	Pegfilgrastim (6 mg) *n* = 422 Placebo *n* = 423	Incidence of grade 3/4 neutropenia	Advanced/ metastatic colorectal cancer	–	4 cycles
Hecht et al. 2010 [231]	RCT	FOLFOX, FOLFIRI, FOIL	Pegfilgrastim (6 mg) *n* = 123 Placebo *n* = 118	Incidence of grade 3/4 neutropenia in first 4 cycles	Colorectal cancer	Pegfilgrastim 62.4* (28–85) Placebo 62.9* (18–87)	17.1 months

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
Clinical trials							
Braess et al. 2009 [232]	Clinical, non-comparative	S-HAM	Pegfilgrastim (6 mg) *n* = 172	Overall toxicity and early death rate	De novo AML	54* (18–83)	13 months
Burstein et al. 2005 [233]	Clinical, non-comparative	AC followed by paclitaxel	Pegfilgrastim (6 mg) *n* = 135	Incidence of FN	Breast cancer	48* (28–71)	4 cycles AC + 4 cycles paclitaxel
Hendler et al. 2011 [234]±	Clinical, comparative	AC followed by paclitaxel	Pegfilgrastim (6 mg) *n* = 57 Filgrastim (300 µg, days 3-10; days 3-7; days 5, 7, 9, 11) *n* = 174	Incidence of FN, hos-pitalisation, dose delays	Breast cancer	52* (27–73) Filgrastim 52* (26-71) 54* (38-69) 48* (28-74)	4 cycles + 12 weeks
Loibl et al. 2011 [235]**	Clinical, non-comparative	Epirubicin, docetaxel, cyclo-phosphamide	Pegfilgrastim (6 mg) *n* = 174	Grade 4 leukopenia	Breast cancer	49* (29–68)	-
Pippen et al. 2011 [236]	Clinical, non-comparative	Dose-dense AC followed by paclitaxel + bevacizumab	Pegfilgrastim (6 mg) *n* = 197	Incidence of treatment-related adverse events	Breast cancer	51.2 ± 9.2	4 cycles + 4 cycles + 10 cycles
Schwartzberg et al. 2009 [237] Cong. abstract	Clinical, comparative	Docetaxel, adriamycine, cyclo-phosphamide	Maxy-G34 (10; 30; 45; 60; 100 µg/kg) *n* = 6; 6; 6; 6; 3 Pegfilgrastim *n* = 8	Duration of severe, grade 4 neutropenia in cycle 1	High risk breast cancer	–	6 cycles
von Minckwitz et al. 2008 [238]##	Clinical, comparative	Docetaxel, doxorubicin, cyclo-phosphamide	Pegfilgrastim (6 mg) *n* = 303 Filgrastim or lenograstim *n* = 374	Incidence of FN	Primary breast cancer	–	6 or 8 cycles

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
Yardley et al. 2010 [239]	Clinical, non-comparative	Gemcitibine, epirubicin and paclitaxel	Pegfilgrastim (6 mg) *n* = 123	Pathological complete response rate	Breast cancer	51* (29–82)	35 months
Miller et al. 2008 [240]	Clinical, non-comparative	Docetaxel and cisplatin with or without BNP7787	Pegfilgrastim (6 mg) *n* = 151	Neuro-toxicity	NSCLC	62* (30–88)	21.7 months
Toppo et al. 2013 [241] congress abstract	Clinical, non-comparative	Dose-dense TCF	Pegfilgrastim (6 mg) *n* = 128	-	Gastro-esophageal cancer	65* (31–81)	44 months
Balducci et al. 2007 [242][§§]	Clinical, non-comparative	Various	Pegfilgrastim (6 mg) *n* = 343 / 73 (PP)	Incidence of FN	Solid tumours/ NHL	72* (65–88)	-
Ozer et al. 2007 [243] sub-analyses: Noga et al. 2007 [244], Rader et al. 2010 [245]	Clinical, non-comparative	Various	Pegfilgrastim (6 mg) *n* = 2,112 *n* = 325 *n* = 971	Hos-pitalisation, dose delay/ reduction	Various NHL Breast cancer	59* ± 12.8 65* (24–93) 54* (19–84)	4-8 cycles Up to 8 cycles
Observational studies							
Chan et al. 2011 [246]	Comparative, observational (retrospective)	Various	Pegfilgrastim *n* = 123 Filgrastim *n* = 81	Incidence of FN	NHL	Pegfilgrastim 55.3 ± 14.8 Filgrastim 56.7 ± 13.1	5.5 cycles Filgrastim 3.9 cycles
Ng et al. 2011 [247]	Non-comparative, observational (retrospective)	CHOP-21 or CHOP-14	Pegfilgrastim (PP/SP) *n* = 132	Incidence of breakthrough FN	Various (80% DLBCL)	55* (49–65)	-

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
Salar et al. 2009 [248] congress abstract	Comparative, observational (prospective)	Various (76% CHOP-R)	Pegfilgrastim (PP/SP) *n* = 127(~100 PP) Filgrastim (PP/SP) *n* = 118 (~84 PP)	Incidence of grade 3/4 neutropenia	NHL (97%) HL (3%)	58* (19–85)	8 cycles or end of chemo-therapy
Hamilton et al. 2013 [249] congress abstract	Comparative, observational (retrospective)	Adjuvant docetaxel/ cyclophosphamide	Upfront pegfilgrastim *n* = 153 No upfront pegfilgrastim *n* = 87	Incidence of FN	Breast cancer	Upfront 57# No upfront pegfilgrastim 52#	–
Jenkins et al. 2012 [250]±±	Non-comparative, observational (retrospective)	Docetaxel, adriamycine, cyclophosphamide	Pegfilgrastim (6 mg) *n* = 263	Incidence of FN	Breast cancer	48* (27–66)	6 cycles
Leung et al. 2012 [251] congress abstract	Comparative, observational (prospective)	58% docetaxel-based chemotherapy	Pegfilgrastim (6 mg) *n* = ~93 Filgrastim (300 µg) *n* = ~47	Incidence and severity of muscle and/or joint pain	Breast cancer	52	2 cycles
Ngamphaiboon et al. 2012 [252]	Non-comparative, observational (retrospective)	TC	Pegfilgrastim (6 mg) *n* = 111	Incidence of FN	Breast cancer	56* (27–79)	19.1 months
Almenar et al. 2009 [253]	Comparative, observational (retrospective)	Various	Pegfilgrastim (PP) *n* = 75 Filgrastim/Lenograstim (PP) *n* = 99; 12	Incidence of CIN/FN, hos-pitalisation, antibiotics	Various	Pegfilgrastim 57.0 ± 14.8 Daily G-CSF 55.4 ± 14.5	–
Almenar-Cubells et al. 2013 [216]	Comparative, observational (retrospective)	Various	Pegfilgrastim *n* = 180 (107 PP, 53 SP, 20 reactive) Filgrastim/Lenograstim *n* = 196; *n* = 15 (78 PP, 50 SP)	Incidence of grade 3/4 neutropenia	Solid tumours (no breast cancer)	Pegfilgrastim: 57.9 ± 13.7 Daily G-CSF: 61.7 ± 12.2	–

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
Heaney et al. 2009 [254]	Comparative, observational (retrospective)	Various	Pegfilgrastim (PP/SP) *n* = 982 Sargramostim (PP/SP) *n* = 982	Infection-related hospitalisation	Various	Pegfilgrastim 58.6 ± 11.5 Sargramostim 57.5 ± 11.6	–
Henk et al. 2013 [255]	Comparative, observational (retrospective)	Various	Pegfilgrastim (PP/SP) *n* = 8569 + 6719 Filgrastim (PP/SP) *n* = 621 + 628 Sargramostim (PP/SP) *n* = 140 + 94	Neutropenia-related and all-cause hospitalisation	Various	Pegfilgrastim 56.1 ± 11.3 Filgrastim 56.6 ± 11.3 Sargramostim 60.0 ± 11.8	–
Hershman et al. 2009 [111]	Comparative, observational (retrospective)	Various	Pegfilgrastim *n* = 721 No G-CSF *n* = 778	Incidence of FN	Solid tumour, lymphoma	–	1 to 8 cycles
Jurczak et al. 2013 [256] congress abstract	Non-comparative, observational (prospective)	Various	Pegfilgrastim (PP) *n* = 1,006	Incidence of FN and safety	Solid tumour, lymphoma	55* (18-86)	–
Morrison et al. 2007 [257]	Comparative, observational (retrospective)	Various	Pegfilgrastim *n* = 1,412 (60% PP) Filgrastim *n* = 1,451 (59% PP)	Describe use of G-CSF, incidence of FN	Solid tumour, lymphoma	–	1 to 8 cycles
Naeim et al. 2013 [258]	Comparative, observational (retrospective)	Various	Pegfilgrastim (PP/SP) *n* = 3,372 Filgrastim (PP/SP) *n* = 163	Risk of hospitalisation	Solid tumour, NHL	Pegfilgrastim 55.1 ± 10.7 Filgrastim 57.5 ± 12.6	–

Author, year	Study design	Chemotherapy regimen	G-CSF interventions (dose)	Primary endpoint	Tumour type	Mean/median* age ± SD (range)	Follow-up (mean)
Tan et al. 2011 [259]	Comparative, observational (retrospective)	Various	Pegfilgrastim (PP/SP) *n* = 4,955 Filgrastim (PP/SP) *n* = 616	–	Various	Pegfilgrastim 57.1 ± 11.6 Filgrastim 58.4 ± 11.0	–
Weycker et al. 2009 [260]	Comparative, observational (retrospective)	Various	Pegfilgrastim (PP/SP) *n* = 14,570 Filgrastim (PP/SP) *n* = 1,193	–	Various	Pegfilgrastim 59* (18–98) Filgrastim 60* (19–90)	1–9 cycles

AC, doxorubicin, cyclophosphamide; AML, acute myeloid leukaemia; CHOP, cyclophosphamide, doxorubicin, vincristin and prednisone; CIN, chemotherapy-induced leukaemia; DLBCL, diffuse large B-cell lymphoma; FN, febrile neutropenia; FOIL, 5-fluorouracil, leucovorin, oxaliplatin, irinotecan; FOLFIRI 5-fluorouracil, leucovorin, irinotecan; FOLFOX, 5-fluorouracil, leucovorin, oxaliplatin; G-CSF, granulocyte-stimulating factor; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; PP, primary prophylaxis; R, rituximab; RCT, randomised controlled trial; SD, standard deviation; S-HAM, high dose cytosine arabinoside and mitoxantrone; SN, severe neutropenia; SP, secondary prophylaxis; TC, docetaxel, cyclophosphamide; TCF, docetaxel, cisplatin, I-folinic acid, 5-fluorouracil

The study design was reported from the perspective of the granulocyte colony-stimulating factor used, e.g. if the study was a randomised controlled trial and patients were randomised according to the chemotherapy regimen and all patients received pegfilgrastim, it was defined as clinical, non-randomised, non-comparative trial. Blank cells indicate that the information was not available.

* not stated in the manuscript if mean or median was reported

Patients with febrile neutropenia received open-label pegfilgrastim

§ 77 additional patients were enrolled and treated open-label with balugrastim

± the three groups receiving different schedules of filgrastim were combined to one 'filgrastim' group

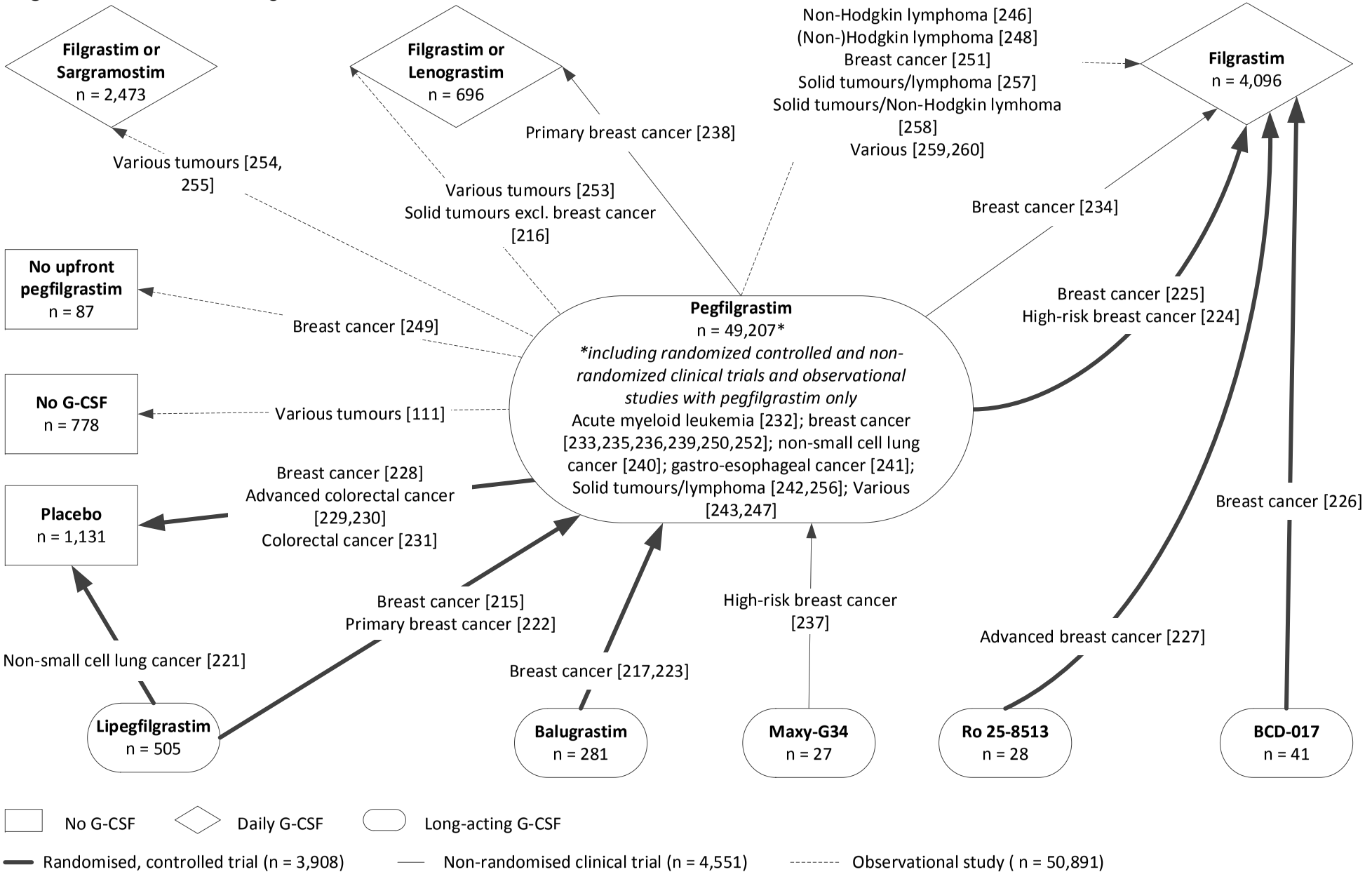
** only the patients randomised to receive pefilgrastim on day two were included

patients receiving concomitant ciprofloxacin (*n* = 567) were excluded from this review

§§ patients receiving pegfilgrastim at physician discretion (*n* = 416) were excluded

±± 185 patients received prophylactic antibiotics

Figure 4.4-2: Network diagram

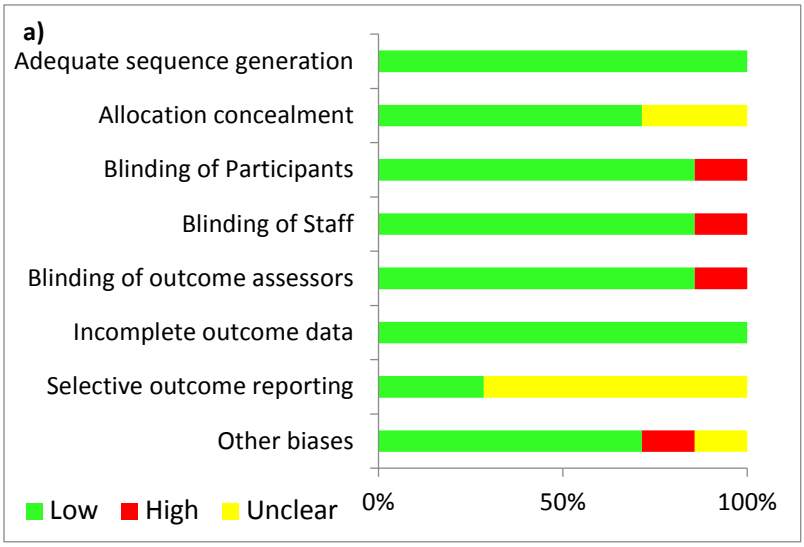


The total number of patients included in randomised controlled trials, clinical trials and observational studies, and in whom a given granulocyte colony-stimulating factor (G-CSF) has been investigated, is stated below the GCSF's or comparator's name. Single-arm studies including pegfilgrastim only are reported in the 'Pegfilgrastim' shape. Comparisons between G-CSFs are indicated by arrows specifying the type of study. The arrows point from the investigated G-CSF to the comparator.

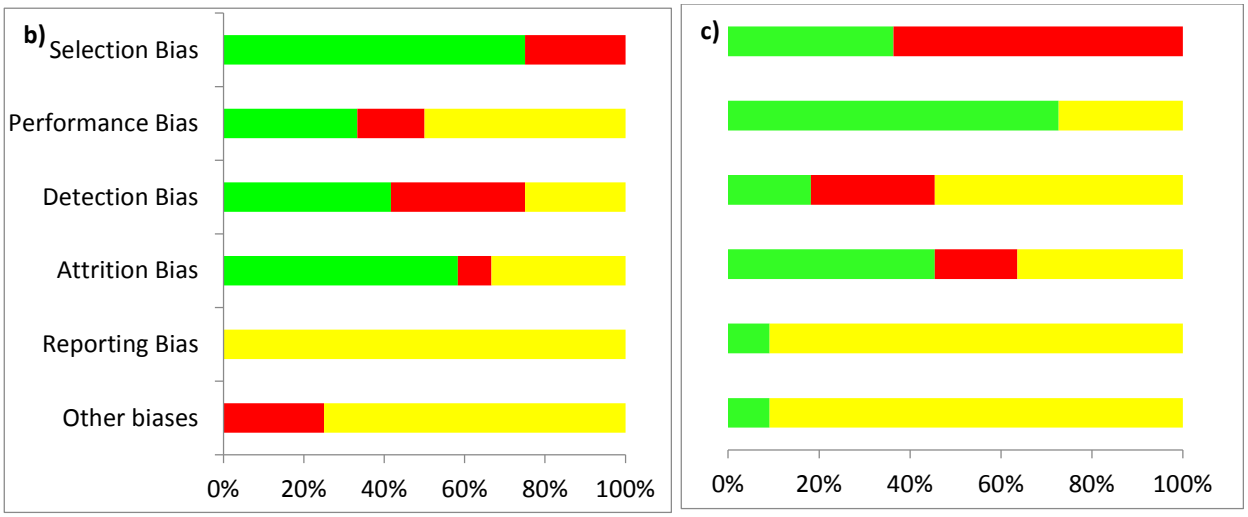
Risk of bias assessment

Risk of bias was typically higher in non-randomized trials and observational studies than in RCTs (Figure 4.4-3). Most studies excluded patients receiving concomitant antibiotic prophylaxis or who had previously received chemotherapy; therefore, risk of performance bias was low. Risk of reporting bias was difficult to assess across all types of studies because the study protocols were not published.

Figure 4.4-3: Risk of bias of included studies



a) Randomised controlled trials (RCTs) are defined as those studies in which patients were randomised to a granulocyte colony-stimulating factor (G-CSF). Adequate sequence generation and allocation concealment refers to selection bias, blinding of participants and staff to performance bias, blinding of outcome assessors to detection bias, incomplete outcome data to attrition bias and selective outcome reporting refers to reporting bias.



b) Non-randomised clinical trials are those in which patients were not randomised to a granulocyte colony-stimulating factor (G-CSF). The risk of bias assessment includes non-randomised clinical trials and observational studies that included more than one granulocyte colony-stimulating factor. **c)** The risk of bias assessment includes non-randomised clinical trials and observational studies that included pegfilgrastim only.

Efficacy and effectiveness of pegfilgrastim

Table 4.4-2 shows efficacy and effectiveness endpoints for studies of pegfilgrastim alone or compared with daily-G-CSFs, placebo or no treatment.

Incidence of FN

Three RCTs reported a significant reduction in FN for pegfilgrastim versus placebo (1% vs 17% [228], 2% vs 6% [229,230], and 2% vs 8% [231]) in patients with breast or colorectal cancer receiving chemotherapy regimens associated with various FN risk profiles. One RCT designed to demonstrate non-inferiority in duration of severe neutropenia reported a significant reduction in FN incidence for pegfilgrastim versus filgrastim (9% vs 18%) in patients with breast cancer [225]. Another RCT with a similar design found a non-significant trend towards lower FN incidence for pegfilgrastim versus filgrastim (13% vs 20%) [224].

Ten clinical trials reported FN incidence across numerous tumor and chemotherapy types, including several dose-dense regimens. In eight of these trials all patients received pegfilgrastim; FN incidence ranged from 1–10% [233,235,236,239-243]. A study in which FN prophylaxis was changed by protocol amendment in subsequent cohorts of patients with primary breast cancer treated with a high FN-risk regimen (docetaxel, doxorubicin and cyclophosphamide), found a significant reduction in the incidence of FN for pegfilgrastim versus daily G-CSF (7% vs 18%) [238]. In contrast, another breast cancer trial in which G-CSF schedules were selected at the physician's discretion reported a higher FN incidence for pegfilgrastim versus filgrastim (11% vs 4%) [234].

Observational studies showed FN incidence was higher among patients with haematological malignancies (14–16%) [246,247] than in those with solid tumors (4–12%) [216,249,250,252,253,256,257]. Five of these observational studies that reported FN incidence compared neutropenia prophylaxis: two studies across various tumor types reported trends towards reduced FN incidence with pegfilgrastim versus daily G-CSF (11% vs 24% and 7% vs 13%, respectively) [216,253], one found a significant reduction (5% vs 7%) [257]; and two did not find a difference for pegfilgrastim versus filgrastim in Non-Hodgkin's lymphoma (NHL) [246] and breast cancer [251]. Significant reductions in FN incidence for pegfilgrastim primary prophylaxis versus no pegfilgrastim primary prophylaxis were also seen in observational studies of patients with breast cancer (4% vs 30%) and in patients with various tumour types (odds ratio [95% confidence interval (CI)]: 0.49 [0.34–0.68]) [111,249],

Incidence of CIN

An RCT in patients with colorectal cancer treated with chemotherapy with a low FN risk (FOLFOX, FOLFIRI or FOIL) found pegfilgrastim significantly reduced CIN incidence compared with placebo (13% vs 43%) [231]. RCTs comparing pegfilgrastim with filgrastim in a non-inferiority setting reported no significant difference in CIN incidence in patients with breast cancer receiving chemotherapy associated with a high FN risk [224,225].

In clinical trials investigating dose-dense regimens, CIN incidence with pegfilgrastim was low and ranged from 3-11% in patients with breast cancer [233,236,239] and 34% in gastro-esophageal cancer [241]. In studies of standard-dose chemotherapy regimens across various tumour types CIN incidence ranged from 22% to 30% [242,243]. One trial reported that pegfilgrastim significantly reduced the incidence of CIN compared with daily G-CSF (37% vs 58%) in patients with breast cancer [238].

Three observational studies reporting CIN incidence compared neutropenia prophylaxis; a difference was not found between pegfilgrastim and filgrastim in patients with breast cancer [251], but in patients with various tumours or NHL CIN incidence was lower in those receiving pegfilgrastim than those receiving daily G-CSF (28% vs 49% and 41% vs 50%) [216,248].

Incidence of hospitalisations due to CIN or FN

One RCT reported a significant reduction in FN-related hospitalisations in patients with breast cancer who received pegfilgrastim versus placebo (1% vs 14%) [228], while another in patients with colorectal cancer found no significant difference in CIN-related hospitalisations [231].

In a clinical trial including patients with various tumour types receiving pegfilgrastim primary prophylaxis in community-based practices in the USA, the incidence of FN-related hospitalisations was 4% [243]. A similar study in elderly patients found the incidence of CIN- or FN-related hospitalisations was 5% [242]. Two clinical trials of patients with breast cancer found no significant difference in incidence and duration of FN-related hospitalisations between pegfilgrastim and daily G-CSFs [234,238].

Three retrospective observational studies enrolling patients with various tumour types found trends towards reduced incidence of hospitalisations due to FN for pegfilgrastim versus daily G-CSF (9% vs 20%, 3% vs 11% and 3% vs 7%) [216,253,258], whereas

another found no significant difference between sargramostim and pegfilgrastim [254]. Two other retrospective observational studies [259,260], reported significant decreases in the risk of CIN-related hospitalisations for pegfilgrastim compared with filgrastim (1% vs 4% and 1% vs 2%); findings supported by a study of two US databases that found pegfilgrastim reduced the risk of neutropenia-related hospitalisation compared with filgrastim [255].

Incidence of chemotherapy dose reductions and delays

In one RCT in patients with breast cancer receiving pegfilgrastim or placebo, there was no significant difference in the proportion of patients receiving their full chemotherapy dose on schedule [228]; however, cross-over from the placebo to the pegfilgrastim arm was allowed if FN occurred. Another RCT in colorectal cancer reported a significant decrease in dose reductions (3% vs 11%) and delays (4% vs 20%) due to neutropenia for pegfilgrastim versus placebo [231].

There was a wide range of incidence of dose delays and reductions in the clinical trials (2%–77% and 2%–33%, respectively), but most papers did not specify whether or not the chemotherapy modifications were due to neutropenia [234,236,241,243]. Only one clinical trial compared the incidence of dose delays (due to FN events and non-haematological toxicity) with pegfilgrastim and filgrastim in patients with breast cancer. It found no significant difference between the two arms [234].

Rates of dose delays and reductions in observational studies also varied considerably between trials (5%–55% and 5%–42%, respectively) [216,246,247,249,252,253]. One study found a significantly lower incidence of delays for pegfilgrastim primary prophylaxis versus no pegfilgrastim primary prophylaxis in patients with breast cancer (5% vs 12%), but found no significant difference in dose reductions [249]. In two studies of patients with various tumour types, fewer dose delays (42% vs 55%) [216] and dose reductions (32% vs 38% and 7% vs 21%) [216,253] due to neutropenia for pegfilgrastim versus daily G-CSF were observed. In a population of Asian patients with NHL, rates of dose reductions and delays were slightly higher in patients who received pegfilgrastim than in those who received filgrastim [246].

Antibiotic use

In one RCT, a non-significant reduction in antibiotic use was reported for pegfilgrastim versus filgrastim (17% vs 21%) in patients with breast cancer [224]. Two RCTs reported a significant reduction in the use of antibiotics due to FN for pegfilgrastim versus placebo,

one in breast cancer (2% vs 10%) [228] and one in colorectal cancer (2% vs 7%) [231].

A clinical trial in breast cancer found no significant difference in the use of antibiotics between patients receiving pegfilgrastim and filgrastim (11% vs 4%) [234].

An observational study found a significant reduction in the use of antibiotics for pegfilgrastim primary prophylaxis versus no pegfilgrastim primary prophylaxis (28% vs 46%) in patients with breast cancer [249]. Two observational studies in patients with various tumour types found lower rates of FN-related antibiotic use in patients who received pegfilgrastim than those receiving daily G-CSF (4% vs 11% and 8% vs 17%); in the former study, this difference reached significance [216,253].

Safety of pegfilgrastim

Table 4.4-2 shows safety endpoints for studies of pegfilgrastim alone or compared with daily-G-CSFs, placebo or no treatment.

All G-CSF-related adverse events

Two RCTs in patients with breast cancer reported that G-CSF-related adverse events (AEs) were similar for pegfilgrastim and filgrastim [224,225]. Another RCT found a non-significant increase in G-CSF-related AEs for pegfilgrastim compared with placebo (11% vs 1%) in patients with colorectal cancer, primarily due to increased bone pain [231]. Pegfilgrastim-related serious AEs were also infrequent (0.5%) in patients with various tumors in a clinical trial [243]. Two observational studies in patients with various tumors reported a non-significant decrease in G-CSF-related AEs for pegfilgrastim versus daily G-CSF (6% vs 10% and 1% vs 5%) [216,253]. None of the studies reported any fatal AEs that were attributed to G-CSF prophylaxis.

Musculoskeletal pain

In two placebo-controlled RCTs including patients with breast or colorectal cancer, occurrence of any-grade musculoskeletal pain was higher in the pegfilgrastim arms than the placebo arms (31% vs 27% and 11% vs 1%) [228,231]. In two further RCTs of patients with breast cancer randomized to pegfilgrastim or filgrastim, overall rates of bone pain were comparable between arms [224,225], and severe bone pain appeared reduced for pegfilgrastim versus filgrastim (1% vs 8%) [224].

In five non-comparative clinical trials, the incidence of any-grade musculoskeletal pain with pegfilgrastim reported ranged from 7% to 26% [233,242] and the incidence of severe

musculoskeletal pain ranged from 0% to 9% [233,236,239,243] across patients with breast cancer and various tumour types.

In general, the reported incidence of musculoskeletal pain was lower in observational studies than in clinical trials. The incidence of any-grade musculoskeletal pain with pegfilgrastim in observational studies varied, from 6% in one study where all patients received pegfilgrastim (with no patients experiencing serious bone or muscle pain) [256], to 50% in patients receiving either pegfilgrastim or filgrastim [251]. In two other observational studies of patients with various tumor types that compared pegfilgrastim with daily G-CSF, bone pain was less common in the pegfilgrastim arms (2% vs 6% and 1% vs 3%) [216,253].

Table 4.4-2: Efficacy, effectiveness and safety of pegfilgrastim

Author, year	G-CSF intervention	Tumour type	Incidence of FN % (95% CI)	Efficacy and Effectiveness				Safety
				Incidence/ duration of neutropenia %/days (95% CI)	Incidence/ duration of hospitalisation %/days (95% CI)	Incidence of chemotherapy delivery parameters % (95% CI)	Incidence of antibiotic use % (95% CI)	Incidence of AEs (%)
RCTs								
Green et al. 2003 [224]	Pegfilgrastim *n* = 77 Filgrastim *n* = 75	High-risk breast cancer	13% vs 20% -7% (-19%, 5%) NS	*grade 4 cycle 1:* 84% vs 83% 0.2 (−0.2, 0.6) days NS	18% vs 31%	Reduction: ~5% had >25% Dose on schedule: 90%	17% vs 21%	All: 57% vs 58% Severe: 1.3% vs 2.7% *Bone pain:* All: 37% vs 42% Severe: 1% vs 8%
Holmes et al. 2002 [225]	Pegfilgrastim *n* = 147 Filgrastim *n* = 150	Breast cancer	9% vs 18% -9% (-17%, -1%) *p* = 0.029	*grade 4 cycle 1:* 77% vs 79%, NS 1.73 vs. 1.76 days NS	–	–	–	Severe: 19% vs 20% *Skeletal pain:* All: 25% vs 26%
Vogel et al. 2005 [228][a]	Pegfilgrastim *n* = 463 Placebo *n* = 465	Breast cancer	1% vs 17% -16% (-19%, -12%) OR 15.0 (6.5, 34.6), *p* < 0.001	–	*Due to FN:* 1% vs 14% OR 12 (5.2, 27.8) *p* < 0.001	Full dose on schedule: 80% vs 78%	*To treat FN:* 2% vs 10% OR 7.5 (3.4, 16.7), *p* < 0.001	*Bone pain:* All: 31% vs 27% Severe: 2% vs 1%
Decaestecker and Pinter et al. 2013 [229,230] Cong. abstracts	Pegfilgrastim *n* = 422 Placebo *n* = 423	Advanced/ metastatic colorectal cancer	2.4% vs 5.7% -3.3% (-6.6%, 0%) OR 0.4 (0.2, 0.9) *p* = 0.014	–	–	–	–	–
Hecht et al. 2010 [231]	Pegfilgrastim *n* = 123 Placebo *n* = 118	Colorectal cancer	2% vs 8% OR 0.3 (0.1–1.0) *p* = 0.04	*Grade 3/4:* 13% vs 43% OR 0.2 (0.1–0.4) *p* < 0.0001	*Due to neutropenia:* 6% vs 8% *p* = 0.55	*Due to neutropenia:* Reduction: 3.3% vs 11%, *p* = 0.02 Delay: 4.1% vs 19.5%, *p* < 0.001	*Due to FN:* 2% vs 7% OR 0.2 (0.1, 1.1) *p* = 0.046	*Bone pain:* All: 10.5% vs 0.9% Severe: 0.8% vs 0%

Author, year	G-CSF intervention	Tumour type	Incidence of FN % (95% CI)	Efficacy and Effectiveness				Safety
				Incidence/ duration of neutropenia %/days (95% CI)	Incidence/ duration of hospitalisation %/days (95% CI)	Incidence of chemotherapy delivery parameters % (95% CI)	Incidence of antibiotic use % (95% CI)	Incidence of AEs (%)
Clinical trials								
Braess et al. 2009 [232]	Pegfilgrastim *n* = 172	*de novo* AML	–	median time to leukocyte recovery (> 1000/uL): 31 days	–	–	–	–
Burstein et al. 2005 [233]	Pegfilgrastim *n* = 135	Breast cancer	1.5%	*Grade 3/4:* 3%	–	Planned dose on time: 88.4%	–	*Musculoskeletal pain:* All: 7%-26% Severe: 5%
Hendler et al. 2011 [234][b]	Pegfilgrastim *n* = 57 Filgrastim *n* = 174	Breast cancer	10.5% vs 4.0%	–	*Due to FN:* 10.5% vs 4.0% 3 days (range: 1–7)	Delay: 3.0% vs 16.1% NS	10.5% vs 4.0%	–
Loibl et al. 2011 [235][c]	Pegfilgrastim *n* = 174	Breast cancer	4.7%	–	–	–	–	–
Pippen et al. 2011 [236]	Pegfilgrastim *n* = 197	Breast cancer	7%	9%	–	*Due to adverse events:* Reduction: 14% Delay: 49.5%	–	*Severe myalgia:* 9% *Severe arthralgia:* 8%
von Minckwitz et al. 2008 [238][d]	Pegfilgrastim *n* = 303 Daily G-CSF *n* = 374	Primary breast cancer	7% vs 18% $p < 0.001$	*Grade 4:* 37% vs 58% $p < 0.01$	*Due to FN:* <1% vs 1% *Due to neutropenia:* 1% vs 1%	–	–	–
Yardley et al. 2010 [239]	Pegfilgrastim *n* = 123	Breast cancer	*Grade 3 FN:* 1%	*Grade 3/4:* 11%	–	–	–	*Severe Myalgia/ Arthralgia:* 7%

Author, year	G-CSF intervention	Tumour type	Incidence of FN % (95% CI)	Efficacy and Effectiveness				Safety
				Incidence/ duration of neutropenia %/days (95% CI)	Incidence/ duration of hospitalisation %/days (95% CI)	Incidence of chemotherapy delivery parameters % (95% CI)	Incidence of antibiotic use % (95% CI)	Incidence of AEs (%)
Miller et al. 2008 [240]	Pegfilgrastim *n* = 151	NSCLC	3.3%	–	–	–	–	–
Toppo et al. 2013 [241] Cong. abstract	Pegfilgrastim *n* = 128	GEC	*Grade 3/4 FN:* 10%	*Grade 3/4:* 34%	–	Reduction: 33% Delay: 77%	–	–
Balducci et al. 2007 [242][e]	Pegfilgrastim *n* = 343	Solid tumors	4% (2%, 6%)	*Grade 3/4:* 30% (25%, 35%)	*Due to neutropenia /FN:* 5% (3%, 7%)	*Due to neutropenia* Reduction: 2% (1%, 4%) Delay: 5% (3%, 7%)	*Due to neutropenia* 10%	*Bone pain:* All: 12% Arthralgia was noted related to pegfilgrastim
	n = 73	NHL	15% (8%, 25%)	*Grade 3/4:* 82% (72%, 90%)	*Due to neutropenia or FN:* 17% (10%, 28%)	*Due to neutropenia* Reduction: 16% (9%, 27%) Delay: 25% (15%, 36%)	*Due to neutropenia:* 55%	
Ozer et al. 2007 [243]	Pegfilgrastim *n* = 2,112	Various	5.6% (4.6%, 6.7%)	*Grade 3/4:* 29.5% (27.6%, 31.5%)	*Due to FN:* 3.5% (3.7%, 4.3%)	*Due to neutropenia* Reduction: 2.9% Delay: 2.1%	*Due to neutropenia:* 5.7% iv, 12.0% oral *Due to FN: 3.6%*	*G-CSF related:* Severe: 0.5% *Musculoskeletal pain: Severe: 0.1%*
Observational studies								
Chan et al. 2011 [246]	Pegfilgrastim *n* = 123 Filgrastim: *n* = 81	NHL	16.3% vs 13.6% *p* = 0.69	–	–	Reduction: 10.6% vs 9.9%, *p* = 1.0 Delay: 18.7% vs 16%, *p* = 0.71	–	–

Author, year	G-CSF intervention	Tumour type	Incidence of FN % (95% CI)	Efficacy and Effectiveness				Safety
				Incidence/ duration of neutropenia %/days (95% CI)	Incidence/ duration of hospitalisation %/days (95% CI)	Incidence of chemotherapy delivery parameters % (95% CI)	Incidence of antibiotic use % (95% CI)	Incidence of AEs (%)
Ng et al. 2011 [247]	Pegfilgrastim *n* = 132	Various (80.3% DLBCL)	13.6%	–	*Due to FN:* CHOP-14: 11.7% CHOP-21: 23.6%	*Due to FN* Reduction/Delay: CHOP-14: 10%/16.7% CHOP-21: 41.7%/19.4%	–	–
Salar et al. 2009 [248] Cong. abstract	Pegfilgrastim *n* = 127 (100 PP) Filgrastim *n* = 119 (84 PP)	NHL (96.6%), HL (3.4%)	PP: 15.8% SP: 21.8%	*Grade 3/4:* 41.1% vs 50.0%	*Due to FN*: 12% 5.9 days vs 12.4 days	Full dose on schedule: 72.1% vs 61.2%	–	–
Hamilton et al. 2013 [249] Cong. abstract	Upfront pegfilgrastim *n* = 153 No upfront pegfilgrastim *n* = 87	Breast cancer	4% vs 30% adj. OR 0.10 $p < 0.0001$	–	11.1% vs 38% adj. OR 0.19 $p < 0.0001$ 2.9 vs 3.8 days	Reduction: 8.5% vs 9.2% OR 0.83, $p = 0.71$ Delay: 4.6% vs 11.5% OR 0.33, $p = 0.046$	28.1% vs 46% OR 0.43, $p = 0.004$	–
Jenkins et al. 2012 [250][f]	Pegfilgrastim *n* = 263	Breast cancer	12%	–	–	–	–	–
Leung et al. 2012 [251] Cong. abstract	Pegfilgrastim *n* = 93 Filgrastim *n* = 47	Breast cancer	No difference between groups	No difference between groups	–	–	–	Over 50% of the patients reported muscle and/or joint pain
Ngamphaiboon et al. 2012 [252]	Pegfilgrastim *n* = 111	Breast cancer	7%	*Grade 3/4:* 9%	*Due to FN*: 6.3%	Reduction: 5% Delay: 5%	0.9% oral	–

Author, year	G-CSF intervention	Tumour type	Incidence of FN % (95% CI)	Efficacy and Effectiveness				Safety
				Incidence/ duration of neutropenia %/days (95% CI)	Incidence/ duration of hospitalisation %/days (95% CI)	Incidence of chemotherapy delivery parameters % (95% CI)	Incidence of antibiotic use % (95% CI)	Incidence of AEs (%)
Almenar et al. 2009 [253]	Pegfilgrastim *n* = 75 (29 PP) Daily G-CSF *n* = 111 (44 PP)	Various	10.7% (5.3%, 19.9%) vs 24.3% (17.2%, 33.1%)	–	*Due to FN:* 9.3% (4.3%, 18.3%) vs 19.8% (13.4%, 28.3%)	*Due to neutropenia* Reduction: 6.7% (2.5%, 15%) vs 20.7% (14.1%, 29.2%)	*Due to FN:* 8% (3.4%, 16.7%) vs 17.1% (11.2%, 25.3%)	*G-CSF-related:* All: 1.3% vs 5.4% *Bone pain:* All: 1.3% vs 2.7%
Almenar-Cubells et al. 2013 [216]	Pegfilgrastim *n* = 180 (107 PP) Daily G-CSF *n* = 211 (78 PP)	Solid tumours except breast cancer	6.7% vs 13.3% *p* = 0.032	*Grade 3/4:* 28.3% vs 49.3% *p* < 0.0005	*Due to neutropenia:* 3.9% vs 14.7% *p* < 0.0005 *Due to FN:* 2.8% vs 10.9% *p* = 0.002	Reduction: 31.6% vs 38.4% *p* = 0.116 Delay: 41.7% vs 54.7% *p* = 0.013	*Due to neutropenia:* 9.4% vs 23.7% *p* = 0.002 *Due to FN:* 4.4% vs 11.4% *p* = 0.013	*G-CSF-related:* All: 6.1% vs 9.5% *p* = 0.219 *Bone/muscle pain:* All: 1.7% vs 6.2% *p* = 0.025
Heaney et al. 2009 [254]	Pegfilgrastim *n* = 982 Sargramostim *n* = 982	Various	–	–	*Due to FN*: 9 vs 4 events *p* = 0.624	–	–	–
Henk et al. 2013 [255]	Pegfilgrastim (reference) *n* = 8,569/ 6,719 Filgrastim *n* = 621/628	Various	–	–	*Due to neutropenia:* HIRD^{SM} database OR 1.8 (1.3, 2.5)/ OptumInsight database OR 2.4 (1.8, 3.1)	–	–	–
Jurczak et al. 2013 [256] Cong. abstract	Pegfilgrastim *n* = 1,006	Various	4%	–	–	–	–	*Bone/muscle/ joint pain:* 6% No serious pain

Author, year	G-CSF intervention	Tumour type	Incidence of FN % (95% CI)	Efficacy and Effectiveness				Safety
				Incidence/ duration of neutropenia %/days (95% CI)	Incidence/ duration of hospitalisation %/days (95% CI)	Incidence of chemotherapy delivery parameters % (95% CI)	Incidence of antibiotic use % (95% CI)	Incidence of AEs (%)
Morrison et al. 2007 [257]	(PP/SP) Pegfilgrastim *n* = 1,412 Filgrastim *n* = 1,451	Various	4.7% vs 6.5% adj. OR 1.4 (1.0, 2.0) p = 0.044	–	–	–	–	–
Naeim et al. 2013 [258]	(PP/SP) Pegfilgrastim *n* = 3,372 Filgrastim *n = 163*	Various	–	–	*Due to neutropenia:* 2.4% (2.1%, 2.7%) vs 6.7% (4.4%, 9.7%) *p* < 0.001	–	–	–
Tan et al. 2011 [259]	(PP/SP) Pegfilgrastim *n* = 4,955 Filgrastim *n* = 616	Various	–	–	*Due to neutropenia :* 1.1% vs 3.5% adj. Or 0.4 (0.2, 0.8) *p* = 0.016	–	–	–
Weycker et al. 2009 [260]	(PP/SP) Pegfilgrastim *n* = 14,570 Filgrastim *n = 1,193*	Various	–	–	*Due to neutropenia:* 1.2% vs 2.1% adj. Or 0.6 (0.4, 1.0) *p* = 0.043	–	–	–

Adj., Adjusted; AEs, adverse events; CI, confidence interval; Cong., congress; FN, febrile neutropenia; G-CSF, granulocyte colony-stimulating factor; GEC, gastro-esophageal cancer; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin's lymphoma; iv, intravenous; NHL, non-Hodgkin lymphoma; NS, not statistically significant; NSCLC, non-small cell lung cancer; OR, odds ratio; PP, primary prophylaxis; SP, secondary prophylaxis; RCT, randomised controlled trial

In the table, the first number corresponds to the upper G-CSF mentioned and the second number corresponds to the lower G-CSF mentioned in the G-CSF intervention column. If available and not otherwise specified, numbers are given in percentages of the patients across all cycles. We also included differences with a 95% CI and/or OR with a 95% CI and/or p-values, if available in the original study. Differences and ORs refer to the upper G-CSF mentioned compared to the lower G-CSF mentioned in the G-CSF intervention column. – indicates that the data was not available.

[a]Patients with FN received open-label pegfilgrastim

[b]The three groups receiving different schedules of filgrastim were combined to one 'filgrastim' group

[c]Only the patients randomized to receive pefilgrastim on day 2 were included

[d]Patients receiving concomitant ciprofloxacin (n = 567) were excluded from this review

[e]Patients receiving pegfilgrastim at physician discretion (n = 416) were excluded

[f]185 patients received prophylactic antibiotics

Other long-acting G-CSFs

Table 4.4-3 shows the efficacy and safety endpoints for studies involving other long-acting G-CSFs.

Lipegfilgrastim

Lipegfilgrastim is pegylated at a different site from pegfilgrastim (threonine 134) using a carbohydrate linker involving two enzymatic steps. In a placebo-controlled RCT in patients with lung cancer, there was no statistically significant reduction in the first-cycle incidence of FN compared to placebo (2% vs 6%) and a significant reduction in the first-cycle incidence of severe neutropenia (32% vs 59%) [221]. G-CSF-related AEs were more common in the lipegfilgrastim arm (14% vs 10%) [221]. In a non-inferiority RCT comparing lipegfilgrastim with pegfilgrastim in patients with breast cancer, there was no significant difference in FN incidence (1% vs 3%), and a non-significant reduction in severe neutropenia incidence (44% vs 51%) [215]. Rates of FN-related hospitalizations and antibiotic use were also comparable between the two study arms (1% vs 2% and 1% vs 3%, respectively) [215]. AEs, including bone pain (14% vs 10%), myalgia (9% vs 6%) and arthralgia (5% vs 2%), were slightly more common with lipegfilgrastim than with pegfilgrastim, but the difference was not significant [215]. In a second RCT in breast cancer, duration of severe neutropenia for lipegfilgrastim and pegfilgrastim was reported to be similar [222].

Table 4.4-3: Efficacy and safety of other long-acting granulocyte colony-stimulating factors

Author, year	G-CSF intervention	Tumour type	Efficacy			Safety
			Incidence of febrile neutropenia % (95% CI)	Incidence/duration of severe neutropenia %/days (95% CI)	Incidence of other efficacy outcomes % (95% CI)	Incidence of all treatment-related AEs %
RCTs						
Bondarenko et al. 2013 [215]	Lipegfilgrastim *n* = 101 (94 PP) Pegfilgrastim *n* = 101 (94 PP)	Breast cancer	*Cycle 1:* 1% vs 3% NS	*Cycle 1:* 43.6% vs 51.1% *p* = 0.341 0.7 vs 0.8 days *p* = 0.126	*Hospitalisation due to FN/ infection* 1% for 1 day vs 2% for 5–6 days *Antibiotic use* 1% vs 3% *Chemotherapy dose:* Delay: 30.7% vs 35.6% Reduction: 0% vs 7.9%	27.7% vs 25.7% *Bone pain-related:* 23.8% vs 16.8% *Bone pain:* 13.9% vs 9.9% *Myalgia:* 8.9% v. 5.9% *Arthralgia:* 5% vs 2% *Serious AEs*: 1% vs 1% *Severe AEs:* 2% vs 1%
Buchner et al. 2011 [222] Congress abstract	Lipegfilgrastim (3; 4.5; 6 mg) *n* = 53; 51; 50 Pegfilgrastim (6 mg) *n* = 54	Primary breast cancer	–	*Cycle 1:* 1.1 days vs 0.8 days vs 0.8 days vs 0.9 days	–	–
Gladkov et al. 2012 [223] congress abstract	Balugrastim (40 mg; 50 mg) Pegfilgrastim (6 mg) *n* = 256	Breast cancer	–	*Cycle 1:* 1.3 vs 1.0 vs 1.2 days NS	–	–
Salafet et al. 2013 [226] congress abstract	BCD-017 (3 mg; 6 mg) *n* = 21; *n* = 20 Filgrastim (5 mg/kg/day) *n* = 19	Breast cancer	5% vs 5% vs 0%	*Cycle 1:* 85.7% vs 65.0% vs 61.1% 0.43 days vs 0.40 days vs 0.33 days NS	–	–

Author, year	G-CSF intervention	Tumour type	Efficacy			Safety
			Incidence of febrile neutropenia % (95% CI)	**Incidence/duration of severe neutropenia %/days (95% CI)**	**Incidence of other efficacy outcomes % (95% CI)**	**Incidence of all treatment-related AEs %**
Viens et al. 2002 [227]	Ro 25-8315 (20; 60; 100 µg/kg) *n* = 9; 9; 10 Filgrastim (5 µg/kg/day) *n* = 8	Advanced breast cancer	0% vs 11% vs 10% vs 0%	–	*Hospitalisations:* 55.6% vs 44.4% vs 60% vs 37.5% *Antibiotic use:* 75% vs 50% vs 60% vs 50% *Chemotherapy dose:* Delay: 1 day	4 vs 4 vs 11 vs 2 events *Bone pain:* 20% *Two episodes of severe bone pain with Ro 25-8315 100 µg/kg*
Volovat et al. 2013 [217]	Balugrastim (40 mg) *n* = 153 (150 PP) Pegfilgrastim *n* = 151 (149 PP) Balugrastim (40 mg, open-label) *n* = 77	Breast cancer	*Cycle 1:* 1.3% vs 2.7% p = 0.446	*Cycle 1:* 58% vs 58.8% 1.1 days vs 1.0 days NS	–	20% vs 19% *Bone pain:* 11.8% vs 10.7% vs 18.2% *Severe AEs:* 19.6% vs 18.7% vs 15.6%
Gladkov et al. 2012 [221]	Lipegfilgrastim *n* = 250 Placebo *n* = 125	Lung cancer	*Cycle 1:* 2.4% vs 5.6% OR 0.39 (0.12–1.26) *p* = 0.1151	*Cycle 1:* 32% vs 59% OR 0.33 (0.21–0.51), p < 0.01 0.6 vs 2.3 days, p < 0.0001	–	*G-CSF-related:* 14% vs 10% *Severe AEs:* 23% vs 18%
Clinical trials						
Schwartzberg et al. 2009 [237] congress abstract	Maxy-G34 (10; 30; 45; 60; 100 µg/kg) *n* = 6; 6; 6; 6; 3 Pegfilgrastim *n* = 8	High-risk breast cancer	2.6% vs 4.2%	*Cycle 1:* 2.2 days vs 1.8 days vs 0.8 days vs 2.2 days vs 1.7 days vs 2.0 days	–	–

AE, adverse event; CI, confidence interval; G-CSF, granulocyte colony-stimulating factor; FN, febrile neutropenia; NS, not statistically significant; OR, odds ratio; PP, per protocol; RCT, randomised controlled trial

In the table, the first number corresponds to the upper G-CSF mentioned and the second number corresponds to the lower G-CSF mentioned in the G-CSF intervention column. If not otherwise specified, numbers are given in percentages of the patients across all cycles. – indicates that the data was not available.

Balugrastim

Balugrastim is a non-pegylated recombinant fusion protein composed of human serum albumin and G-CSF harvested from yeast. It has been investigated at a dose of 40 mg in two RCTs in patients with breast cancer treated with doxorubicin and docetaxel. In one, incidence (58% vs 59%) and duration (1.1 days vs 1 day) of severe neutropenia in cycle 1 were similar for balugrastim and pegfilgrastim [217]. There was no significant difference in FN incidence in cycle 1 between balugrastim and pegfilgrastim (1% vs 3%). The frequency of treatment-related AEs was similar for balugrastim and pegfilgrastim (20% vs 19%) [217]. The second RCT found similar durations of severe neutropenia for balugrastim and pegfilgrastim (1.3 days vs 1.2 days) [218].

BCD-017, Maxy-G34 and Ro 25-8315

BCD-017 (empegfilgrastim), Maxy-G34 and Ro 25-8315 are all covalent conjugates of recombinant human G-CSF and polyethylene glycol. Small RCTs compared BCD-017 and Ro 25-8315 with filgrastim in patients with breast cancer but found that neutropenia-related outcomes, including rates of FN, were generally lower in the filgrastim arms [226,227]. Safety data were reported in the Ro 25-8315 study and suggest G-CSF-related AEs are more common with Ro 25-8315 than with filgrastim [227]. Maxy-G34 was compared with pegfilgrastim in a clinical trial. The incidence of FN and duration of CIN were similar in the two study arms [237]. No safety data were reported.

Discussion

To our knowledge, this is the only systematic review of long-acting G-CSFs that includes newly developed agents and data from both clinical trials and observational studies. We identified 12 RCTs, 12 clinical trials and 17 observational studies, including 58,342 patients in total. Studies in patients with breast cancer were dominant, partly because these were the registration studies for the G-CSFs.

Pegfilgrastim studies included a range of patient populations, cancer types and stages, and chemotherapy regimens. Efficacy and effectiveness results were generally consistent. Although pegfilgrastim did not uniformly show better efficacy or effectiveness in all studies, the vast majority showed better efficacy or effectiveness compared to daily G-CSF, no upfront pegfilgrastim, no G-CSF or placebo in terms of reduction of the incidence of CIN (4/7 studies), FN (11/14 studies), chemotherapy dose delays and reductions (6/8 studies), antibiotic use (6/7 studies) and neutropenia-related hospitalizations (9/13 studies). The

observed variation may be partly explained by differences in patient populations and cancer types, or in the way G-CSF was administered. Thirteen (35%) studies of pegfilgrastim reported safety data and most of these focused on musculoskeletal pain; only three studies reported other G-CSF-related AEs. This suggests that the safety profile of G-CSFs may be generally accepted and studies now investigate only specific AEs known to be associated with their use. The incidence of G-CSF-related AEs was similar between pegfilgrastim and filgrastim. The incidence of bone pain and severe bone pain was lower or no different for pegfilgrastim than filgrastim in most RCTs and observational studies (4/6 studies).

Previously published systematic reviews and meta-analyses of RCTs comparing pegfilgrastim with daily G-CSF or placebo by Cooper et al. and Pinto et al. found that pegfilgrastim more effectively reduced the incidence of FN [261,262]. The RCT reported by Decaestecker et al. and Pinter et al. [229,230], showing better efficacy for pegfilgrastim than placebo in reducing the incidence of neutropenia in colorectal cancer patients, reported in this systematic review was not included in these previous systematic reviews. We additionally included non-randomized clinical trials and observational studies that have not been included in former systematic reviews [261,262]. Nevertheless, the results of our systematic review are generally consistent with these studies. However, while well-designed RCTs have a low risk of bias, inclusion criteria can be restrictive. The observational studies included in our review indicate an advantage for pegfilgrastim over daily G-CSFs or no treatment, suggesting that the efficacy of pegfilgrastim demonstrated in clinical trials has been translated into clinical practice. In fact, we found a greater magnitude of reduction in CIN incidence with pegfilgrastim versus filgrastim in observational studies than RCTs; this could be due to a shorter duration of G-CSF use in current practice (e.g. 5–6 days in clinical practice vs 10–11 days in clinical trials) [98]. Importantly, the safety data from observational studies were consistent with data from RCTs, suggesting that the pegfilgrastim safety profile can be used to guide treatment in a broad patient population. However, care should be taken when interpreting the results of observational studies, owing to the higher risk of bias and confounding factors.

Almost all the studies including other long acting G-CSFs were RCTs of patients with breast cancer (7/8 studies) receiving doxorubicin and docetaxel (5/8 studies). Lipegfilgrastim has been the most extensively tested (3/8 studies) and appears to be similar to pegfilgrastim regarding the reduction in duration of severe neutropenia in patients with breast cancer.

Efficacy of lipegfilgrastim in reducing the incidence of FN was not statistically superior to placebo in a congress abstract describing an RCT in patients with lung cancer [221]. Lipegfilgrastim has now been approved in Europe for reducing the incidence and duration of FN in adults with cancer who are receiving cytotoxic chemotherapy [211]. Further clinical and observational studies in a wider range of tumor types and chemotherapy regimens will confirm whether its efficacy and safety is maintained across a broader patient population in real-world clinical practice. Balugrastim has also been investigated in two phase 3 RCTs of patients with breast cancer and has an efficacy and safety profile comparable to that of pegfilgrastim. Again, further studies will determine whether this translates to other patient populations. Notably, the incidence of FN in the pegfilgrastim arms of the lipegfilgrastim and balugrastim studies (3% in cycle 1 for both studies [215,217]) was lower than in the registrational pegfilgrastim studies (9% and 7% in cycle 1 [224,225]), despite a similar study design and patient population. Maxy-G34 also appears to be non-inferior to pegfilgrastim; however, it was tested in only a very small number of patients (n = 35) [237]. BCD-017 and Ro 25-8315 did not appear to be as effective at reducing the incidence of FN as filgrastim [226,227].

Because very few studies reported long-term outcomes of G-CSF use and two systematic reviews by Kuderer and Lyman et al. [263,264] looking at survival have previously been published, we did not include overall survival as an endpoint. In 2007, Kuderer et al. [263] published a systematic review of infection-related and early mortality during chemotherapy by type of G-CSF. They reported that there is insufficient data to draw conclusions. An updated analysis in 2013 by Lyman et al. [264] concluded that all-cause mortality is reduced in patients receiving chemotherapy with primary G-CSF support. However, Lyman et al. did not report results by type of G-CSF. We are still awaiting long-term survival data for the newer long-acting G-CSFs. Future research should examine in more detail the effects of long-acting G-CSFs on survival outcomes.

As is true for all systematic reviews, the validity of our findings is limited by the quality of its underlying studies. Another limitation is that some studies did not report how many patients received primary prophylaxis versus secondary prophylaxis. This may have led to an underestimation of effectiveness. Furthermore, the studies were not all consistent in their definitions of FN and CIN and the number of chemotherapy cycles over which they reported data. Finally, combined measures of effect are missing in our analysis.

It is clear that pegfilgrastim is widely used in clinical practice across a broad patient

population. Lipegfilgrastim and balugrastim were similar to pegfilgrastim in reducing the duration and incidence of CIN and FN in five studies. Furthermore, the safety profiles of the recently-developed long-acting G-CSFs were comparable to pegfilgrastim based on the phase 3 studies identified by this systematic review. These G-CSFs may prove to be valuable therapeutic options; however, there is a need for further studies in broader patient populations to confirm their effectiveness and safety in real-world clinical practice. New biosimilar G-CSFs and next-generation drugs targeting the G-CSF receptor are also in the early stages of development [212] and should be assessed against the current standard of care.

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Authors' contributions

All authors were involved in the design of the study. KA was responsible for the first draft of the protocol, which was critically reviewed, further developed and approved by all authors. KA performed the literature search, collected and extracted the data. AMP was responsible for the risk of bias assessment and the first draft of the manuscript. KA and AMP were responsible for the second draft. All authors contributed to data interpretation, critically reviewed all manuscript versions and approved the final version.

Conflicts of interest

A. M. Pfeil's institution of employment receives unrestricted scientific/educational grants from Amgen. K. Allcott is an employee of Oxford PharmaGenesis™ Ltd, which has received project funding from Amgen. R. Pettengell received honoraria from Amgen and Roche. G. von Minckwitz receives research funding from Amgen and Teva and served on an advisory board for Amgen. M. Schwenkglenks's institution of employment receives unrestricted scientific/educational grants from Amgen and he has served on advisory boards for Amgen. Z Szabo is an employee of Amgen and owns stock and stock options in the company.

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5 General discussion

This work is based on four published studies based on observational data that addressed the epidemiology of CLL (4.1), the description of the epidemiology of neutropenia and febrile neutropenia including risk factors (4.2), the development and external validation of a risk prediction model for febrile neutropenia (4.3), and a systematic literature review on the efficacy of prophylactic measures for neutropenia (4.4).

Our studies showed that primary care data from the UK CPRD seemed to be valid to determine the epidemiology of CLL, but unsuitable to evaluate the occurrence of neutropenia. In the hospital cohort of breast cancer patients, predictors of FN were blood parameters and genetic factors. The apparent predictive ability of the model was better than chance, but the model lacks external validation. In external validation of a risk prediction model in NHL patients, predictive performance of the model was only slightly lower than in the training dataset. Several options for preventing CIN or FN are available. If successful risk prediction models are developed, these may help to optimally target prophylaxis with G-CSFs to those patients at high risk of FN. Every study has its own discussion section reporting the limitations and strengths of the study designs and study findings.

This general discussion puts the findings and contributions of the conducted studies in a broader context. It further reports the public health relevance and the implications of the studies taking into account other studies conducted in this field of research. Recommendations for ongoing and future studies are provided. Conclusions from the studies this work was based on are reported in a separate section following the general discussion section.

5.1 Incidence of chronic lymphocytic leukaemia and neutropenia

The majority of the population-based incidence rates of CLL reported in other studies are out of date. Several European studies were found that reported CLL incidence rates, but these incidence rates were calculated before the year 2010 [168,169]. Therefore, many studies addressing CLL-related topics repeatedly report CLL incidence rates of 3-5/100’000 people or person-years. For some readers, this might suggest that CLL incidence rates did not change over the last 10 years. In contrast, a trend of increasing CLL incidence rates over the last 10 years was observed in the study conducted by the author. This observation is supported by a European study that also found an increase in

the incidences of leukaemias [57] and by reports from the UK [169].

The analysis in the study conducted by the author covers the time period from 2000 to 2012. Until 2008, the diagnosis of CLL relied on the National Cancer Institute Working Group criteria that have been introduced in 1996. New guidelines developed by the International Workshop on CLL (IWCLL) redefined CLL diagnosis criteria [265]. Molica et al. and Call et al. recently reported that using the new diagnosis guidelines, less early CLL cases were diagnosed [266,267]. Instead, patients that would have been diagnosed with early CLL according to the National Cancer Institute criteria were re-classified as monoclonal B-cell lymphocytosis, where patients can have a CLL phenotype, but no CLL symptoms such as lymphadenopathy or an enlarged spleen [268]. If the change in diagnosis criteria was not immediately implemented in clinical practice, but with a short delay, it could explain the temporary decrease in CLL incidence rates between the years 2010 and 2011 observed in the UK CPRD. Because in the other years between 2000 and 2012, an increase in CLL incidence rates was observed.

Overall, the UK CPRD database was a valid approach to calculate CLL incidence rates for the UK. The International Lymphoma Epidemiology Consortium [269] aims to investigate the etiologies of NHL subtypes including CLL. Although the Consortium's primary goal is to establish risk factors for the numerous subtypes of NHL, this could be an important future source to obtain nation-wide incidence rates of CLL. Published results by the Consortium include several European countries, but not Switzerland. In Switzerland, the cantonal cancer registry data are compiled by the Foundation National Institute for Cancer Epidemiology and Registration (nicer, http://www.nicer.org/de/). This cancer data are not publicly available. Nevertheless, summary statistics can be obtained from the nicer website and detailed information may be available upon request.

As this is the first worldwide study assessing medical resource use in CLL patients, the study findings cannot be compared to other studies. During the time period from 2000 to 2012, average medical resource utilisation per year in terms of number of recorded referrals and hospitalisations increased significantly. The observations were in line with key statistics of the UK National Health Service for the general population [175]. Possible reasons for the increase were explained in section 4.1 and included changes in the recording approach by GPs, changes in clinical practice patterns, the need for immediate treatment for some CLL patients. According to Molica et al. and Call et al., the change in diagnosis criteria led to a reduction in the time to first treatment of CLL patients [266,267].

This may be an additional reason why the number of referrals and hospitalisations recorded in the UK CPRD increased that explains part of the increase.

As mentioned in the study, the reported medical resource utilisation may only cover part of the total medical resource utilisation of CLL patients. CLL patients are often treated with chemotherapy, which is applied in secondary or tertiary care. CLL patients with chemotherapy-related complications such as infections or neutropenia are often hospitalised. This part of the medical resource utilisation may not be reflected in the primary care database. A recent study in elderly CLL patients of the USA using Medicare and a combination of linked databases showed that elderly patients often received chemotherapy with chlorambucil, rituximab, fludarabine or a combination of these chemotherapy drugs [270]. The study reported in this work also recorded prescriptions for CLL patients, but in comparison to the US study only few prescriptions for chemotherapy overall and no prescriptions for rituximab were recorded. Although the CPRD records referrals to specialists and hospitalisations, detailed information about these patient visits is missing. Linking the CPRD primary care database to other databases such as the hospital episode statistics may provide a more comprehensive overview on the medical resource utilisation of CLL patients.

The author's study (4.1) reported in this work is the most recent study that assessed population-based CLL incidence rates and to our knowledge, it is the first study assessing medical resource utilisation in CLL patients. Further studies assessing the trends in incidence rates of CLL should consider the change in diagnosis criteria of CLL when interpreting the study findings. Linkages to other than primary care databases may provide a better estimate of medical resource utilisation in CLL patients.

5.2 Risk factors and risk prediction model of febrile neutropenia occurrence

Several international guidelines base the initial assessment of the risk of CIN or FN on the applied chemotherapy regimen [98,141-143] conveying the impression that the applied chemotherapy regimen is a key predictor of CIN or FN occurrence. Based on that, the guidelines recommend the prophylaxis with G-CSFs if the risk of developing FN is more than 20%. The findings of the study conducted by Weycker et al. support the fact that the applied chemotherapy regimen determines a substantial part of the risk for FN occurrence [93]. They showed that prophylaxis with G-CSFs and/or antibiotics was mainly given to cancer patients receiving a chemotherapy regimen with a high or intermediate risk of

developing FN [93]. In these patients, G-CSF prophylaxis was given up to 75% of the patients, whereas in patients at low risk of FN G-CSF or antibiotic prophylaxis was given to about 20%. The guidelines recommend that patient risk factors are taken into account when the risk of developing FN with the planned chemotherapy regimen is intermediate (10-20%) [98,141].

To date, numerous studies reported risk factors of CIN and FN including patient-related, chemotherapy-related and tumour-related factors [104-109,111-120]. Blood count-related risk factors such as WBC, RBC, ANC or haemoglobin were the most commonly reported risk factors. A recent study by Lyman et al. summarised the available evidence on risk factors of FN and published a systematic literature review on risk factors for FN [110]. Some studies also reported protective factors [105,106,111,115]. Based on these studies, G-CSF prophylaxis and chemotherapy dose reductions or dose delays that occur before an FN event are effective in reducing the risk of FN. More recently, genetic factors were shown to be significant predictors of FN in various tumour types [121-126]. These risk factors have not yet been confirmed in multiple studies.

Most of the reported studies above have in common that they focused on patient-related, chemotherapy-related and tumour-related factors or genetic factors. Those studies that included genetic factors only adjusted their estimate for certain patient-related and tumour-related factors, but not for other risk factors that have been reported and published before [121-126]. One study this work is based on developed a risk prediction model for the occurrence of FN in breast cancer patients including patient-related, chemotherapy-related, tumour-related characteristics and genetic factors. Not all of the previously published risk factors that were included in this study could be confirmed. Probably, some patient-related or tumour-related factors did not remain in the model, because the included genetic factors masked the effect of these risk factors. Another explanation could be that risk factors of FN that were reported in e.g. patients with haematological cancers or colon cancer patients do not apply to breast cancer patients and vice versa. This may challenge the development of one single risk prediction model that can be used for all types of cancers. Of the numerous genetic factors included as potential predictors of FN risk, two were identified that were associated with a very high risk of FN in a small subset of the study population. Before using these factors in decision-making on G-CSF prophylaxis in this subset of patients can be considered, the findings need to be confirmed by other studies.

Although several risk factors including genetic factors were combined in the study of this work, the predictive ability of the model was only acceptable and comparable to the predictive performance of former published studies not including genetic factors [105,106,113,127,130]. Possible reasons could be that some important previously reported risk factors or protective factors such as G-CSF prophylaxis and chemotherapy dose modifications could not be addressed in the model. All published models do not reliably identify patients that are likely to develop FN, but reliably identify patients that are unlikely to develop FN. Although it may be of benefit to know which patients probably do not need G-CSF prophylaxis, further refinement of these models to improve the PPV is necessary. Lyman et al. [110] published a comprehensive overview of risk factors for FN reported in the literature that could be helpful in designing future risk prediction models.

A recently published risk prediction model by Pastor et al. used another approach to predict the occurrence of prolonged high grade neutropenia in cancer patients undergoing chemotherapy [271]. The authors developed a pharmacokinetic and pharmacodynamic model that described the neutrophil time course in cancer patients undergoing chemotherapy [272]. Based on this model, the authors derived decision rules for the prophylactic application of G-CSFs [271]. The model only included ANC measures at different time points of the chemotherapy cycle as a predictor of FN risk. Predictive performance of the model was good with a resulting area under the ROC curve of 0.875. Because blood count data are routinely measured in cancer patients undergoing chemotherapy, this model approach could complement other model development approaches. The combination of ANC counts at different time points and patient-related, chemotherapy-related, tumour-related and genetic factors may improve the predictive performance of the risk prediction model in external validation to an extent such that the model can be useful in clinical practice.

The risk prediction models of neutropenia presented in this work need further refinement to improve the predictive ability of the models. Based on the results of the above presented models, the impact of genetic factors that are associated with multidrug resistance or metabolism of chemotherapy drugs on the occurrence of CIN or FN in cancer patients should be further evaluated. Another approach to refine neutropenia risk prediction models is to determine the impact of frailty including comorbidity on the occurrence of neutropenia.

Another important step towards the development of an international applicable neutropenia risk prediction model would be the combination of available datasets in cancer patients

looking at neutropenia occurrence. If the collected data would be comparable and could be combined to a single, comprehensive dataset, it might further improve the predictive ability of risk prediction models in neutropenia. The resulting risk prediction model should be externally validated in an independent dataset. Optimally, by performing a prospective study that applies the resulting risk scores to patients undergoing chemotherapy to help identify patients at low or high risk of neutropenia.

5.3 External validation of a risk prediction model of febrile neutropenia occurrence

Internal validity of a risk prediction model can be determined quite easily using the same population as the model was developed in. Several techniques are available such as split-sample validation, cross-validation and bootstrapping that help to determine if the model does what it was intended to do. But internal validation does not provide evidence about the generalisability of the model of interest. External validity is a measure of how the model works in an independent population or other settings.

External validations of risk prediction models in general are rare [201]. Jenkins et al. performed a partial validation of their original model [104] using an independent dataset [108]. The original predictive model was developed in breast cancer patients and included the ANC and the absolute lymphocyte count. In addition, the Jenkins' model [104] was externally validated by other researchers. Chen et al. tested the Jenkins' model in breast cancer patients without pre-specifying successful validation criteria [135]. They concluded that the Jenkins' model did not accurately identify patients at high risk of FN and that the model should be expanded by including the absolute monocyte count [135].

Predictive ability in the external validation of the model of FN occurrence in NHL patients undergoing chemotherapy was lower than the pre-defined successful validation criteria. Therefore, the model was judged inappropriate to be used in clinical practice. Other studies also observed a slight decrease in model performance in internal validation [105,106,113,127,130] and therefore, it was not surprising that we observed a decrease in external validation. Possible reasons could be the differences in the variable definitions or the difference in population size of the two data sources. Although the two databases were quite comparable, some variables available in the training dataset were not available in the validation dataset and vice versa. In addition, the smaller study was used for the model development and the bigger study for the model validation. On the one hand, it would speak in favour of the validity of the developed model if it could successfully predict FN

occurrence in the bigger population. On the other hand, the model performance in external validation could have been better with the model being developed in the bigger population. Nevertheless, probably neither of the models would have been applicable for clinical use.

Several risk prediction models for CIN and FN based on risk factors have been developed. Up to now, no model is validated and clinically used. The observed differences in model performance under different conditions, especially the decrease in model performance using an independent dataset, confirm the importance of external validation before risk models are put to clinical use [134]. If the prediction and external validation is successful, further steps about the implementation of the risk score in clinical practice should be initiated.

As mentioned above, validated risk models would only be of clinical use if they supported physicians' treatment decisions [207]. Ideally, the results of a prediction model should be transferred to a simplified risk score. The resulting risk score should be as straightforward as possible to be easily implemented into clinical practice. Clinicians are probably not willing to apply the risk score in their daily practice if it takes too much time to evaluate the individual risk for FN of a patient based on the risk score. Therefore, it is of utmost importance that clinicians are involved early in the development of a potential risk score. The results should be disseminated in collaboration with clinicians. Other researchers and public health systems should get access to the risk prediction score to increase awareness of the potential use of the score and to improve it on an ongoing basis.

Furthermore, it is also important to highlight that the aim of risk prediction models is not to replace the decision-making process of clinicians. When assessing the risk of CIN or FN in a cancer patient undergoing chemotherapy, the experience of a clinician adds substantial information to the assessment of patient-related and chemotherapy-related factors. Instead, a risk score should be a reliable support for the clinician to make the decision about the administration of supportive measures or not.

5.4 Systematic literature review of granulocyte colony-stimulating factors

Previously published systematic reviews and meta-analyses on the efficacy of G-CSFs only included randomised controlled trials (RCTs) and concluded that G-CSFs are effective and safe for the prophylaxis of FN [261,262]. Recently, a narrative review of G-CSFs as prophylaxis of FN was published [273]. All systematic reviews showed consistent results regarding the efficacy of G-CSFs as prophylaxis against neutropenia. However, efficacy

results very often do not tell us if G-CSFs have the same effect under real-life conditions. There is growing evidence in the literature that efficacy does not directly translate into effectiveness because issues of generalisability and external validity are seldom considered [274,275].

Compared to these systematic reviews, the review included in this work also considered clinical trials and observational studies and still came up to the same conclusions. A disadvantage of this approach is that the risk of bias in clinical trials and observational studies is higher than the risk of bias in RCTs. In RCTs, patients are randomly assigned to an intervention or a comparator, whereas allocation does not have to be at random in clinical trials or is not at random in observational studies. This may introduce bias and confounding. Therefore, the findings from observational studies should be carefully interpreted. On the other hand, results from observational studies are more generalisable. In RCTs, G-CSFs are used in an ideal setting according to guidelines or a pre-defined protocol. Observational studies more appropriately reflect the use of G-CSF in clinical practice.

In clinical practice, there is a wide variability in the use of G-CSFs [276,277]. Many patients receive GCSFs not according to guideline recommendations. Sometimes GCSFs are not administered although recommended by guidelines and vice versa [278]. A literature review showed that the administration of prophylactic daily G-CSFs such as filgrastim not according to guideline recommendations led to compromised patient outcomes due to CIN or FN [273]. In cases where prophylactic G-CSF has not been assigned and cancer patients develop FN, it has been shown that appropriate FN management plays an important role to avoid further complications. The study by Meisenberg et al. showed that concordance with guidelines, staff and patient education, and implementation of standardised treatment pathways for FN improve FN management [279].

There are other supportive options available to prevent the occurrence of CIN, FN or related complications in cancer patients. Antimicrobial prophylaxis has been recommended by the American Society of Clinical Oncology if patients are at risk of prolonged neutropenia or other neutropenia-related complications and infections that increase the risk of mortality [144]. Studies showed that antimicrobials are effective in treating FN [280,281] and time to antibiotic administration may influence mortality [282]. Correct antibiotic or antifungal administration can be essential when treating neutropenia-related infections [100,282]. The issue with antibiotic prophylaxis is the potential of

antibiotic resistance, which does not apply to G-CSFs. One downside of G-CSFs is that they are costly [283]. But if risk prediction models can be successfully validated and support clinical decision-making, they may improve the cost-effective use of G-CSFs.

Overall, G-CSFs substantially impacted on the prophylaxis and treatment of neutropenia. Although available G-CSFs such as filgrastim and pegfilgrastim have been successful and are commonly used, numerous new biosimilar G-CSFs are in development. Companies are also interested in developing other G-CSF agonists that activate the G-CSF receptor in a different way which could lead to an additive effect or increased efficacy of G-CSFs [212].

Even with the development of new-acting G-CSFs, it might be challenging to outreach the already achieved efficacy of G-CSFs [212]. In this case, harmonisation of guidelines and clear recommendations for the use of G-CSFs in clinical practice are another important step to translate efficacy of RCTs into clinical practice and increase the effectiveness of G-CSFs [212].

5.5 Implications

In research in general, the availability of data and access to appropriate data sources is essential. Often, epidemiological studies are conducted using sub-samples of the populations of interest, e.g. patients visiting a specific health care provider or patients being enrolled in a specific study. These population sub-samples may not be representative of the entire population of interest and may lead to biased estimates. More reliable data sources for the assessment of incidence or prevalence rates of certain diseases could be nationwide primary care databases such as the CPRD in the UK or nationwide disease registries such as cancer registries in e.g. the Netherlands, Switzerland or USA. Depending on the type of disease, primary care databases or disease registries are more appropriate. For example, chronic diseases such as diabetes are monitored by primary care physicians and can be captured in primary care databases. For diseases where referrals to specialists or hospitals are often such as cancer, disease registries may provide a more comprehensive picture of the disease.

Risk prediction models or risk scores are developed to help to identify patients at high risk of a certain event or outcome. In regard to the successful development of these tools and the application in clinical practice, external validation is necessary. As it is difficult to get access to independent datasets, research policies should be in place that support data

sharing among researchers. Ideally, financed research programs should be available that allow the systematic validation of promising risk prediction models and risk scores in independent datasets. In order to facilitate collaboration among researchers, these research programs should be initiated by the government and gradually be handed over to research councils that guide and monitor the systematic validation in independent datasets.

To achieve an evidence-based and efficient management of patients, research results regarding risk prediction models and risk scores should be disseminated and available to the public. If a successful risk score has been developed and validated, recommendations or guidelines for patient management set up by the government in collaboration with researchers and clinicians may anticipate implementation in clinical practice. Further, standardisation of patient management approaches may improve patient outcomes.

6 Conclusions

Our studies showed that primary care databases can be used to calculate incidences of cancer and obtain medical resource utilisation of cancer patients in primary care. In only few cases, the database recorded information on the type of chemotherapy, on neutropenia occurrence, and on the use of G-CSFs. Linkage to databases from secondary and tertiary care settings would be necessary to obtain details about chemotherapy and derive the incidence of neutropenia in cancer patients.

Neutropenia in cancer patients undergoing chemotherapy is a relevant clinical issue. Depending on the chemotherapy regimen applied and the presence of other potential risk factors of neutropenia, the risk of CIN and FN varies. Evaluating the impact of genetic factors on neutropenia occurrence in cancer patients is a younger field of interest. Therefore, further studies including genetic factors are necessary.

Adding genetic factors did not much improve the predictive performance of the neutropenia risk prediction model presented in this work. The limited predictive ability of the model in the training dataset showed that further evaluation of risk factors is necessary. Because predictive performance of another model was lower in external validation, careful clinical decision making until validated models are available with adequate predictive ability is of utmost importance. There is a clear need for further studies and continuing validation of proposed risk predictors and tools.

Validated prediction models with adequate predictive ability may help to optimally target prophylaxis with G-CSFs to those patients at high risk of CIN or FN. Several daily and long-acting G-CSFs such as filgrastim, lenograstim, pegfilgrastim, balugrastim and lipegfilgrastim are available that reduce the incidence of CIN, FN and related hospitalisations or antibiotic use. The development of new valuable supportive options is ongoing. In times of increasing health care costs and scarce resources, the cost-effective use of supportive measures may become necessary.

Availability of and access to appropriate data sources are necessary to develop and validate risk prediction models to target G-CSF prophylaxis to patients at high risk of FN as has been shown by the studies this work is based on. These studies further contribute to the development of an evidence-based, efficient and cost-efficient approach to prevent neutropenia in cancer patients.

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8 Appendix

8.1 Supplementary material for 4.4

Table 8.1-1: Detailed search strategy

	Searches EMBASE	Hits
#1	exp recombinant granulocyte colony stimulating factor/ or filgrastim.mp.	12170
#2	pegylat*.mp.	12216
#3	1 and 2	392
#4	($pegfilgrastim or SD01 or neulasta or neulastim or imupeg).mp.	1150
#5	(PEG-rmetHuG-CSF or polyethylene glycol-conjugated filgrastim).mp.	2
#6	(balugrastim or CG-10639 or lipegfilgrastim or XM-22 or glycopegylated C-CSF).mp.	5
#7	(lonquex or neugranin or albugranin or SPI-2012 or LAPS-GCSF or HM10460A).mp.	6
#8	(Extimia or BCD-017).mp.	0
#9	3 or 4 or 5 or 6 or 7 or 8	1381
#10	exp cancer chemotherapy/ or exp chemotherapy/ or chemotherapy.mp.	491608
#11	cancer.mp. or exp neoplasm/	3447398
#12	(tumor or tumour).mp.	1939478
#13	10 or 11 or 12	3783824
#14	exp neutropenia/ or neutropenia.mp. or exp febrile neutropenia/ or exp severe congenital neutropenia/	73934
#15	9 and 13 and 14	734
#16	limit 15 to (human and English language)	578
	Search Cochrane Library	**Hits**
#1	MeSH descriptor: [Granulocyte Colony-Stimulating Factor] explode all trees	1018
#2	*filgrastim	653
#3	pegylat*	959
#4	(#1 or #2) and #3	34
#5	$pegfilgrastim or SD01 or neulasta or neulastim or imupeg	136
#6	PEG-rmetHuG-CSF or polyethylene glycol-conjugated filgrastim	1
#7	balugrastim or CG-10639 or lipegfilgrastim or XM-22 or glycopegylated C-CSF	144
#8	lonquex or neugranin or albugranin or SPI-2012 or LAPS-GCSF or HM10460A	0
#9	Extimia or BCD-017	0
#10	#4 or #5 or #6 or #7 or #8 or #9	302
#11	chemotherapy	31372
#12	MeSH descriptor: [Neoplasms] explode all trees	44664
#13	cancer or oncology	73625

#14	MeSH descriptor: [Neutropenia] explode all trees	
#15	tumor or tumour	20482
#16	#11 or #12 or #13 or #14 or #15	91759
#17	MeSH descriptor: [Neutropenia] explode all trees	1346
#18	neutropenia	3855
#19	#17 or #18	3855
#20	#10 and #16 and #19	82
	Searches MEDLINE & MEDLINE InProcess	**Hits**
#1	exp Granulocyte Colony-Stimulating Factor/	28724
#2	$filgrastim.mp.	3198
#3	pegylat*.mp.	12216
#4	(1 or 2) and 3	442
#5	($pegfilgrastim or SD01 or neulasta or neulastim or imupeg).mp.	1150
#6	(PEG-rmetHuG-CSF or polyethylene glycol-conjugated filgrastim).mp.	2
#7	(balugrastim or CG-10639 or lipegfilgrastim or XM-22 or glycopegylated C-CSF).mp.	5
#8	(lonquex or neugranin or albugranin or SPI-2012 or LAPS-GCSF or HM10460A).mp.	6
#9	(Extimia or BCD-017).mp.	0
#10	5 or 6 or 7 or 8 or 9	1159
#11	chemotherapy.mp.	459728
#12	cancer.mp. or exp Neoplasms/	3447398
#13	oncology.mp. or exp Medical Oncology/	143668
#14	(tumor or tumour).mp.	1939478
#15	11 or 12 or 13 or 14	3795647
#16	neutropenia.mp. or exp Neutropenia/	73934
#17	10 and 15 and 16	675
#18	limit 17 to (human and english language)	532

Table 8.1-2: Definitions of outcome measures

Outcome	Definition	Used by
Febrile neutropenia	- oral temperature ≥ 38°C and ANC <0.5x10^9/L	[208,209,221,227]
	- oral temperature ≥ 38°C for more than 1h and ANC <0.5x10^9/L	[147,230,231,246,249]
	- oral temperature ≥ 38.2°C and ANC <0.5x10^9/L	[220,235,250,253,261]
	- oral temperature ≥ 38.3°C and ANC <0.5x10^9/L	[147,210,246,249]
	- oral temperature ≥ 38.5°C and ANC <0.5x10^9/L	[227,230,236]
	- oral temperature ≥ 38°C and ANC <1.0x10^9/L	[209,215,264]
	- oral temperature ≥ 38.2°C and ANC <1.0x10^9/L	[229,253]
	- oral temperature ≥ 38.3°C and ANC <1.0x10^9/L	[246]
	- oral temperature > 38.5°C and ANC <1.0x10^9/L	[264]
Neutropenia	- Grade 3/4 or severe: ANC <1.0x10^9/L	[143,208,209,227,229,246,253,254]
	- Grade 4 or severe: ANC <0.5x10^9/L	[143,209,220,235,246,253,262]
Hospitalisation	- due to infection ICD-9-CM 001.x-139.x	[228]
	- due to febrile neutropenia ICD-9-CM 288.0 and ICD-9-CM 780.6	[228]
	- neutropenia ICD-9-CM 288.0 or fever ICD-9-CM 780.6	[231,247]
Chemotherapy dose delivery	- dose reductions (≥15% of standard doses)	[106,208,210,250]
	- (1-dose received/dose planned)%	[249]
	- dose reduction (<80% of chemotherapy dose in cycle 1)	[209]
	- dose delays (≥7 days)	
	- dose delays (>3 days)	[209,250]
	- full dose on schedule (≤15% dose reduction and ≤3 days dose delay)	[106,208,210,249]
	- planned dose on time (≥80% of planned dose and ≤3 days dose delay)	[254] [261]
Adverse events	CTCAE v.3.0	[143,234,242,251]
	CTCAE V.4	[250]
	WHO toxicity criteria	[261]

ANC, absolute neutrophil count; CTCAE, Common Terminology Criteria for Adverse Events; ICD, International Classification of Diseases, Ninth Revision, Clinical Modification; WHO, World health organization

Curriculum Vitae

Personal data

Name	**Alena Maria Pfeil**
Date of birth	20.01.1987
Nationality	Swiss
Citizenship	Speicher (AR)
Marital status	longtime partnership, no children

Education

2010 - 2014	PhD in Epidemiology at the Institute of Pharmaceutical Medicine (ECPM), University of Basel *Neutropenia in cancer patients, risk prediction models of neutropenia, and supportive measures*
2010	Master's degree in Human Biology at the University of Zurich *A cross-sectional survey to evaluate knowledge, attitudes and practices (KAP) regarding seasonal influenza vaccination among international travellers*
2008	Bachelor's degree in Biology at the University of Zurich
2001 - 2005	Grammar school, Kantonsschule Romanshorn *Biology and Chemistry*

Professional/Teaching activities

Since 2014	Assistant lecturer, Faculty of Science, University of Zurich *Clinical epidemiology and quantitative research in health care*
Since 2014	Assistant lecturer, Faculty of Medicine, University of Basel *Interprofessional Projects in Medicine, Wissenschaftsmonat, Seminar on neutropenia in cancer patients*
Since 2012	Reviewer for scientific journals *e.g. Annals of Oncology, Breast Cancer Research and Treatment, BMC Public Health, Supportive Care in Cancer, PharmacoEconomics*
Since 2011	Website maintenance of the Impact of Neutropenia in Chemotherapy European Study Group (INC-EU)
Since 2011	Part-time research associate (50%) at the Institute of Pharmaceutical Medicine (ECPM), University of Basel

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Continuing education

2014	27th Residential Summer Course in Epidemiology *Epidemiological Methods and Statistical Models in Epidemiology, Clinical epidemiology*
Since 2013	Postgraduate training in medical product development, European Center of Pharmaceutical Medicine (ECPM)
Since 2011	Postgraduate training in Public Health and Health Economics, Swiss School of Public Health plus (SSPH+), Universities of Basel, Bern, Zurich, Lausanne, Geneva, Lucerne, Neuchatel and Lugano *Statistical Methods for Epidemiology; Ethics in Biomedical and Public Health Research; Introduction to STATA; Epidemiological data analysis – Advanced Methods for Exposure-Response Modeling; SSPH+ summer school in Public Health Policy, Economics and Management; Epidemiology winter school – Survival analysis; Applied Bayesian Statistics in Medical Research; Systematic Reviews and Meta-Analysis – a practical approach; Multilevel Modeling – Analysis of clustered data; Writing a journal article – and getting it published; Logistic Regression*
2011 - 2014	Courses in transferable skills *Scientific Writing Course (Paul Skandera), Presentation courses (Michael J. Vivion, ECG Inc.; Susanne Matuschek, Matuschek Consulting), Fund raising course (Andrea Degen, EUrelations AG)*
2011 - 2014	Courses of the University of Basel *Key Issues in Drug Discovery and Development, Basic Biostatistics I+II, Statistical Modelling, Epidemiological Concepts, Key Issues in International and Public Health, Health Systems, Health financing and health economic evaluations*

Internship

2008	Internship at the Institute of Clinical Chemistry and Haematology at the Canton Hospital St. Gallen

Skills

Languages	German: mother tongue English: fluent (FCE 2005, CAE course ongoing) French: basic knowledge
Computer	Broad software skills including statistical packages STATA, SPSS

Grants

2013	ISPOR congress and travel supported by the Swiss Association for Health Economics (SAG/ASE)

Publication list

Peer-reviewed publications

Pfeil AM, Imfeld P, Pettengell R, et al. Trends in epidemiology and medical resource use in patients with chronic lymphocytic leukaemia: insights from the UK Clinical Practice Research Datalink (CPRD). *Ann Hematol. 2014; [Epub ahead of print]*

Pfeil AM, Allcott K, Pettengell R, et al. Efficacy, effectiveness and safety of long-acting granulocyte colony-stimulating factors for prophylaxis of chemotherapy-induced neutropenia in patients with cancer: a systematic review. *Support Care in Cancer 2015; 23(2): 525-545*

Vulsteke C, **Pfeil AM**, Schwenkglenks M, et al. Impact of genetic variability and treatment-related factors on outcome in ealry breast cancer patients receiving (neo-) adjuvant chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide and docetaxel. *Breast Cancer Research and Treatment* 2014; 147(3): 557-570

Pavic M, **Pfeil AM**, Szucs TD. Estimating the potential annual welfare impact of innovative drugs in use in Switzerland. *Front Public Health* 2014; 2:48

Pfeil AM, Vulsteke C, Paridaens R, et al. Multivariable regression analysis of febrile neutropenia occurrence in early breast cancer patients receiving chemotherapy assessing patient-related, chemotherapy-related and genetic risk factors. *BMC Cancer* 2014; 14(1):201

Gutzwiller FS, **Pfeil AM**, Colet JC, et al. Determinants of Quality of Life of Patients with Heart Failure and Iron Deficiency Treated with Ferric Carboxymaltose: FAIR-HF sub-analysis. *Int J Cardiol* 2013; 168(4):3878-83

Schwenkglenks M, Bendall KL, **Pfeil AM,** et al.: External validation of a risk model of febrile neutropenia occurrence in non-Hodgkin lymphoma patients. *Leuk Lymphoma* 2013; 54(11):2426-32

Lothgren M, Ribnicsek E, Schmidt L, Habacher W, Lundkvist J, **Pfeil AM**, et al. Cost per patient and potential budget implications of denosumab compared with zoledronic acid in adults with bone metastases from solid tumours who are at risk of skeletal-related events: an analysis for Austria, Sweden and Switzerland. *Eur J Hosp Pharm* 2013; 0:1-5

Szucs TD, **Pfeil AM**: A Systematic Review of the Cost Effectiveness of Herpes Zoster Vaccination. *PharmacoEconomics* 2013; 31(2):125-136

Bollhalder L, **Pfeil AM**, Tomonaga Y, Schwenkglenks M: A systematic literature review and meta-analysis of randomized clinical trials of parenteral glutamine supplementation. *Clin Nutr.* 2013; 32(2):213-23

Pfeil AM, Kressig RW, Szucs TD. Alzheimer's dementia: a budget-impact and cost-utility analysis of a combination treatment of a cholinesterase inhibitor and memantine in Switzerland. *Swiss Medical Weekly* 2012; 142:w13676

Pfeil A, Mütsch M, Hatz C, Szucs TD: A cross-sectional survey to evaluate knowledge, attitudes and practices (KAP) regarding seasonal influenza vaccination among European travellers to resource-limited destinations**.** *BMC Public Health* 2010; 10:402

Non-peer-reviewed publications

Pettengell R, Schwenkglenks M, **Pfeil AM**. Neutropenia in cancer treatment – familiar but not resolved. ScienceOmega Reviews UK 2013 Issue 1.

Pettengell R, Schwenkglenks M, **Pfeil AM**, Leonard R. Comment submitted on NICE draft guideline "Neutropenic sepsis: prevention and management of neutropenic sepsis in cancer patients".

Presentations

Oral presentations

2014	diss:kurs, Universität Basel, Basel, Switzerland *Neutropenia in cancer patients can be fatal – can we predict it and optimally target prophylactic measures*
2014	TV Sendung "praxis gsundheit akut", Bern, Switzerland *Neue Medikamente – Nutzen für Gemeinwohl* *http://www.santemedia.ch/de/gesundheitspolitische-sendungen.1194/2011-12-13.1273/neue-medikamente-nutzen-fur-gemeinwohl.2114.html*
2014	Weiterbildung zum Krankenhausfachapotheker, Österreichische Apotheker-kammer, Vienna, Austria *Interpretation of pharmacoeconomic studies*
2012	Advisory Board Meeting, Merz, Vancouver, Canada *Budget-impact and cost-utility analysis of a combination treatment in Alzheimer's dementia*
2012	Swiss Advisory Board, Novartis, Basel, Switzerland *Economic input to Alzheimer's dementia*
2011	9th Impact of Neutropenia in Chemotherapy European Study Group (INC-EU) Meeting, Zurich, Switzerland

Poster presentations

2013	European Cancer Congress (ECCO/ESMO), Amsterdam, Netherlands *The impact of baseline and genetic parameters on progression free survival and overall survival in breast cancer patients receiving neoadjuvant or adjuvant chemotherapy with fluorouracil, epirubicin and cyclophosphamide. Eur J Cancer 2013; 49(Suppl. 2): S477*
2013	Swiss Public Health Conference, Zurich, Switzerland *Literature review on potential clinical and economic effects of eHealth, barriers to implementation and role of incentives*
2012	Alzheimer's Association International Conference, Vancouver, Canada *Economic evaluation of the combination therapy of a cholinesterase inhibitor and memantine in Alzheimer's dementia in Switzerland. Alzheimer's & Dementia 2012; 8(4): P387*
2009	Swiss Public Health Conference, Zurich, Switzerland *A cross-sectional survey to evaluate knowledge, attitudes and practices (KAP) regarding seasonal influenza vaccination among international travellers*