Risk Management of Biologicals:
A Regulatory and Clinical Perspective

Thijs J. Giezen

The research presented in this PhD thesis has been conducted under the umbrella of the Regulatory Science collaboration between the Dutch Medicines Evaluation Board (MEB) and the Utrecht Institute for Pharmaceutical Sciences (UIPS). The MEB is dedicated to ensure that licensed medicinal products during their whole life-cycle have a positive be-nefit-risk. This role requires intensive collaboration with academic and clinical partners in order to develop new assessment and decision making methods, to engage with the clinic and to strengthen regulatory science. This PhD thesis aims to go beyond its scien-tific merits as such by delivering science, learning and insight to promote public health.

Risk management of biologicals:
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Risicomanagement van biologicals vanuit een regulatoir
en een klinisch perspectief
(met een samenvatting in het Nederlands)

Proefschrift

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THIJS JAN GIEZEN

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PROMOTOREN: Prof. dr. H.G.M. Leufkens
                     Prof. dr. A.C.G. Egberts

CO-PROMOTOREN: Dr. A.K. Mantel-Teeuwisse
                     Dr. S.M.J.M. Straus

Voor mijn ouders

Contents

Chapter 1

General introduction

Historical perspective of biological drug innovation

Biologicals, also called biopharmaceuticals, are defined by the European Medicines Agency (EMA) as products which are produced by or extracted from a biological source and that need for its characterisation and the determination of its quality a combination of physico-chemical-biological testing together with the production process and its control.[1] According to the first part of the definition currently used by the EMA, also products extracted from trees and plants already in use at the time of Hippocrates (400 before the common era) can be considered biologicals. For example, lotions derived from distilled bark and leaves of willow trees which were used to alleviate pain.[2] After the long use of these products it was the chemist Felix Hoffman in 1897 who was able to extract from the willow tree and synthesize acetylsalicylic acid and thereby created a pure form of the substance. One of the first man-made drugs, salvaran, a treatment for syphilis, was produced by Paul Ehrlich in the beginning of the 1900s. Sulphonamide, which was discovered in 1931, was the first chemically synthesized drug.[2] It was, however, discovered a couple of years later that sulphonamide was metabolized in the human body into its active ingredient sulphanilamide. Sulphanilamide had been widely used in the dye-making industry since 1906 but its anti-microbial activity was identified some decades later.[3]

Even before the discovery of sulphonamide in the 1920s insulin was already available for the treatment of patients suffering from diabetes mellitus. Insulin is the first well-known and widely used biological, which was directly extracted from pigs by Banting and Best.[4,5] Although this biological was available for the treatment of patients, the majority of drugs were synthesized chemically throughout most of the twentieth century.[2] After the chemical revolution the introduction of recombinant DNA and hybridoma technologies in the 1980s enabled the large-scale production of complex protein based drugs, like enzymes, hormones, monoclonal antibodies, and receptors by the end of the century. This resulted in new treatments for a variety of chronic, e.g. rheumatoid arthritis and Crohn's disease, and sometimes life-threatening diseases such as cancer.[6,7] The number of biologicals on the market now numbers just over 200 products.[8] Today's practice of medicine would be unthinkable without the availability of these compounds that have shaped important innovative therapy options.[6,7]

In addition, biologicals have become important in pharmaceutical innovation; they represented 24% and 22% of all new chemical entities approved by the US and EU regulatory authorities between 2003 and 2006, respectively.[8] In addition, the number of billion dollar-plus biologicals has increased from six in 2002 to 22 in 2007 and about 20% of all blockbuster drugs in 2007 involved biologicals.[9] Previous research has focused on the economic evaluation and diffusion of biologicals in clinical practice.[10] The economic evaluation is considered important for biologicals because of their high costs; in 2005 12.9% of the spending on pharmaceuticals in the USA concerned biologicals whereas these products only counted for 6.3% of all molecules available.[11] A report published in the Netherlands in 2000, suggested a 20% increase per year in the in-hospital use of expensive drugs, of which biologicals are an important part.[12] Although the costs of biologicals are high, these products have found their way in clinical practice as important treatment options, as described previously. However, some serious safety concerns have been identified with these agents.[13-18]

Chapter 1

Characteristics of biologicals and their relation with safety

Biologicals have specific characteristics compared to the traditional small molecule drugs and therefore may carry specific risks (Table 1). The main safety challenges of biologicals can be classified in three categories: 1) related to molecules, production and purification, 2) extrapolation from pre-clinical and clinical testing and, 3) nature of the adverse drug reactions (ADRs).

Table 1: Differences between biologicals and small molecules and examples of safety related problems related to these differences.[6,7,21,24,31]

Biologicals versus small molecules	Examples of safety related problems
Large complicated molecules and often mixtures of different isoforms	-
Relatively unstable/ complex production and purification process/ (small) changes in manufacturing process can influence safety	Formation of aggregates can influence the immunogenic potential Pure red cell aplasia in patients treated with epoetin alpha following manufacturing changes
Manufactured in living cells	The host cell used and contamination with host cell DNA and host cell material can influence the immunogenic potential, e.g. natural interleukin-2 was reported to be less immunogenic than interleukin-2 produced by *Escherichia Coli*
Potential for immunogenicity	Thrombocytopenia after treatment with recombinant thrombopoetin due to neutralizing antibodies blocking endogenous thrombopoetin
Limited predictability of pre-clinical to clinical data due to species-specific action and immunogenicity to human proteins in animals	Cytokine storm in TeGenero phase I trial Human interferon has a different pharmacological effect as mouse interferon in mice
Adverse events often related to the pharmacology	Tuberculosis with the use of the TNF-α inhibitor infliximab

In contrast to the chemically synthesised small molecules, biologicals are very complex and large molecules (Figure 1). In addition, biologicals have a very complex production and purification process involving multiple steps with the risk of influencing the characteristics of the end product at every single step of the production cascade.[19] This can be illustrated by the clear increased incidence of pure red cell aplasia (PRCA) in patients treated with a particular formulation of human epoetin-α, which occurred post-1998. In this new formulation human serum albumin was replaced with glycine and polysorbate 80. Prior to 1998 only four cases of PRCA were associated with human epoetin.[20] This example illustrates that vigilance is very important for biologicals in case changes in the production process are introduced. PRCA was the result of an immunogenic reaction to the administered epoetin and the endogenous available protein. The immunogenicity of a biological can be influenced by a variety of factors including issues related to the production process. The following characteristics can influence the immunogenic potential: 1) the host cell used, e.g. *Escherichia Coli*-derived interferon-β has been linked to higher immunogenicity due to a lack of glycosylation compared to Chinese hamster ovary derived interferon-β;[21] 2) the type of disease, e.g. antibody production may be increased by

infectious diseases or reduced by immunosuppression; 3) the dose frequency, e.g. immunogenicity increases with more frequent dosing; 4) the risk of contamination of the end product with host cell DNA and host cell material.[22]

Figure 1. Molecular weight of biologicals and small molecules.

Cumulative %

Figure 1. Molecular weight of biologicals and small molecules.
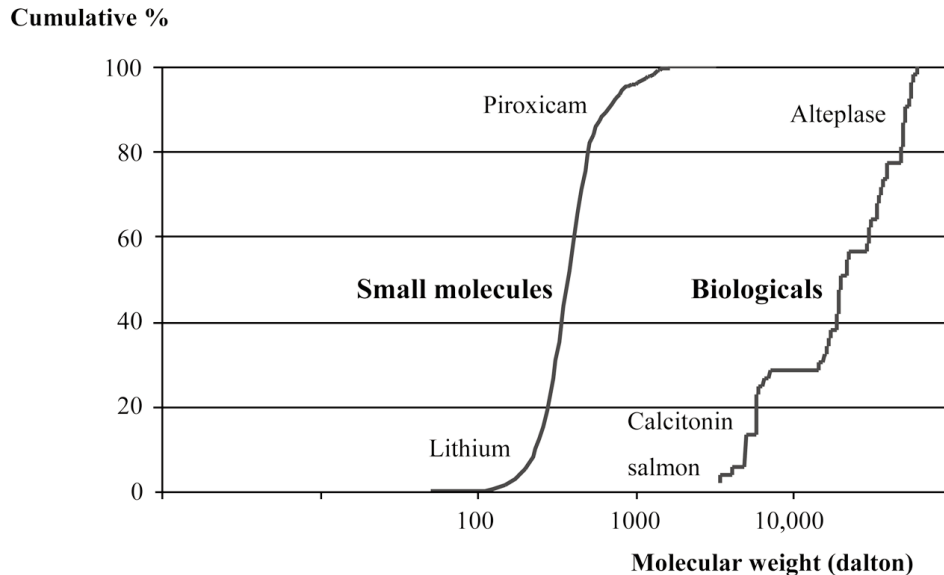
Predictability of pre-clinical data to humans is limited for biologicals due to species-specific actions and immunogenic properties in animals.[23] To obtain valuable results from the preclinical (toxicology) studies, a relevant test animal should not only be selected based on pharmacological activity and low immunogenicity, but suitable pharmacokinetic factors also should be taken into account.[24,25] The limited predictability of pre-clinical to clinical data can be clearly illustrated by the cytokine storm, which occurred during the first-in human study of the super agonist anti-CD28 monoclonal antibody TGN1412. The cytokine storm was not observed during the animal studies.[17] It is expected that these factors will result in limited knowledge on the safety profile at the moment of marketing giving pharmacovigilance and pharmacoepidemiology an important role to further study the safety profile of biologicals.

Toxicity of biologicals can often be attributed to the pharmacological activity,[24] as illustrated by the occurrence of the opportunistic infection tuberculosis with the use of inhibitors of tumour necrosis factor-α (TNF-α).[26,27] Another example includes the occurrence of progressive multifocal leukoencephalopathy (PML) with the use of certain monoclonal antibodies. The occurrence of PML has already been linked to certain diseases in which the patients were immunocompromised, e.g. HIV infection, and might also be related to certain biologicals with a strong immunosuppressive mode of action.[28] Mode of action driven safety assessment will therefore be of growing importance in the safety

assessment of biologicals, which is expected to also play a role in the pharmacovigilance planning for these products. In addition to the toxicity based on the pharmacological activity, products containing biologicals are specifically prone to the induction of immunogenicity. Although immunogenicity is not clinically relevant in many cases, antibodies can neutralize the effect of the biological and therefore lead to a loss of efficacy and can influence the safety profile. Consequences on safety can be classified as acute consequences, for example anaphylactic reactions, non-acute consequences, for example delayed type hypersensitivity reactions, and cross-reactivity with an endogenous counterpart.[29] The increased incidence of pure red cell aplasia in patients treated with a particular formulation of epoetin-α as well as the occurrence of a severe thrombocytopenia in patients treated with a megakaryocyte-derived growth factor was the result of antibodies that cross-reacted with an endogenous counterpart.[21,22,30,31]

Pharmacovigilance and risk management of biologicals

Knowledge on the full safety profile of a drug is limited at the moment of marketing due to the limitations of randomized controlled trials, including, among others, a limited sample size and duration and a homogeneous population.[32] In addition, regulators are more and more challenged by the need to balance rapid market access for new drugs with the wish for comprehensive safety data.[33] For biologicals, it is expected that certain characteristics, including the limited predictability of pre-clinical to clinical data and the fact that half of the biologicals approved in the USA are designated orphan drugs limiting preapproval experience,[34,35] further limit knowledge on the full safety profile at the moment of marketing for these drugs. Post-marketing collected safety data offers a valuable and necessary complement to the clinical trials.[32] Pharmacovigilance is defined by the World Health Organization (WHO) as the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other drug-related problem.[36] The need for pharmacovigilance is underlined by the findings of a study in the US in which 10.2% of all new molecular entities approved between 1975 and 1999 required a post-marketing issued black box warning or were withdrawn due to safety reasons.[37]

Pharmacovigilance activities can consist of a variety of activities including both routine activities and more pro-active activities.[38] Routine pharmacovigilance, including spontaneous reporting of adverse drug reactions (ADRs), has an important function in the detection of new, rare and/ or serious ADRs.[39] However, as widely described and acknowledged, underreporting is high and expected to be in excess of 90%[39] and causality assessment to establish a relation between a drug and an ADR remains difficult.[40] Causality assessment may be confounded by a variety of factors including concomitant diseases, genetics, severity of disease, and medication. In addition, a plausible temporal relationship between intake of the drug and occurrence of the event is a factor that is usually taken into account during causality assessment as well.[41] For biologicals, multiple difficulties related to spontaneous reporting of ADRs are identified and can be expected. Biologicals are often indicated to treat severe and/ or life-threatening diseases.[7] Patients treated with biologicals are, therefore, often suffering from multiple diseases and are treated with multiple drugs, which may hamper adequate causality assessment. The possible relation

between use of infliximab and the occurrence of lymphoma in patients suffering from in-flammatory bowel disease has, for example, been confounded by concomitant use of other immunosuppressive agents.[42] In addition, the relation between intake of the drug and occurrence of the adverse event is often difficult to assess. This can be illustrated by a published research letter in which a potential relation between use of TNF-α inhibitors and occurrence of leukaemia was described. Exposure time until the diagnosis of leukaemia differed between a few months up to several years.[43]

In the past years, a more pro-active approach towards the identification and quantification of safety data has been anticipated as an important step for improvement.[44,45] At this moment there is broad agreement among regulators and industry that the collection of safety data should be a continuous process.[33] This has been anticipated by the implementation of guidelines for risk management programmes.[46] In the EU marketing applicants are obliged to submit an EU-Risk Management Plan (EU-RMP) for all marketing applications of new chemical entities including biologicals and biosimilars since November 2005.[38,47] As already described previously, the predictability of pre-clinical to clinical data is limited for biologicals and these agents are relatively new. Learning in the post-marketing phase is therefore even more important for biologicals.

An EU-RMP summarizes the results of the performed pre-clinical and clinical trials, laid down in the safety specification, and proposes pharmacovigilance activities and inter-ventions designed to identify, characterise, prevent or minimise risks relating to medicinal products. Also the effectiveness of these interventions should be evaluated (Box 1).[38,47]

Safety specifications can be classified as identified risks (adequate evidence of an asso-ciation with the medicinal product), potential risks (there is a basis for suspicion of an association with the medicinal product but the association has not been confirmed), or missing information including populations not studied in the pre-authorisation phase.[35,45]

The pharmacovigilance activities proposed in the EU-RMP comprise both routine and additional activities (Post-Authorisation Safety Studies (PASS)).[38] PASS is defined as a pharmacoepidemiological study (non-interventional study) or a clinical trial (interven-tional study) carried out in accordance with the terms of the marketing authorisation, con-ducted with the aim of identifying or quantifying a safety hazard relating to an authorised medicinal product.[48] The requirements for an EU-RMP are similar for biologicals and small molecules. However, due to the different characteristics between biologicals and small molecules differences in both identified issues as well as approaches for further monitoring might be expected.

The data sources that can provide adequate information for PASS are anticipated to be different between biologicals and small molecules. Compared to small molecules biolog-icals are often used in a specialised (hospital) setting or, as is the case in the Netherlands, for some biologicals the drug is directly delivered to the patient without intervention of the community pharmacy where the patient is registered.[49] Large-population based data-bases, often including general practitioners and community pharmacy data are therefore expected to include limited data on biologicals. Pharmacovigilance data will, therefore, have to be obtained from different sources. Disease and exposure registries are expected to be a valuable data source to study exposure data and safety of biologicals. The value

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Box 1: Content of the EU Risk Management Plan.[37]

Part I
- A safety specification: summarises the safety profile of the medicinal product at the particular point in time of its life-cycle
- A pharmacovigilance plan: pharmacovigilance activities should be described to further study (potential) safety concerns

Part II
- An evaluation of the need for risk minimisation activities, and if there is a need for additional (i.e. non-routine) risk minimisation activities
- A risk minimisation plan

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of these registries has, for example, already been established for the post marketing safety evaluation of biologicals for patients suffering rheumatoid arthritis. A Danish registry covered approximately 90% of all patients treated with biologicals. A twenty-fold increase of non-serious adverse events and a doubling of the serious adverse events was registered compared to the mandatory reports to the Danish Medicines Agency.[50] The German registry RABBIT, in which rheumatoid patients treated with biological agents are included, was used to study the association between use of TNF-α inhibitors and the occurrence of Herpes Zoster infection. It was found that the monoclonal antibodies adalimumab and infliximab had a significant higher risk for Herpes Zoster infections compared to the conventional disease-modifying anti-rheumatic drugs.[51] In addition to registries, pooling of data from randomised controlled clinical trials has also shown to serve as a valuable tool to assess adverse events of these agents. This value can be illustrated by the observed 3.3 fold increased risk for malignancies and the 2.0 fold increased risk for serious infections in patients treated with tumour necrosis factor antagonists after pooling of clinical trial data.[32]

It can be concluded from the above that specific challenges in the pharmacovigilance and pharmacoepidemiology of biologicals exist.

Objectives of this thesis

The subject of this thesis is the risk management of biologicals. This will be studied from both a regulatory and a clinical perspective. There are two main objectives. The first objective is to investigate the main characteristics of post-marketing identified adverse drug reactions of biologicals and activities for the identification of these adverse drug reactions. The second objective is to identify risk factors and early markers for an early identification of patients at risk for a certain adverse drug reaction.

Outline of this thesis

This thesis contains seven studies described in three chapters.

Chapter 2 focuses on the main characteristics of regulatory activities and actions in the pharmacovigilance of biologicals. In *chapter 2.1* the characteristics of post-authorisation safety studies are evaluated, with regard to nature of the safety concerns, completeness of the study protocols, and data source to be used, in the first cohort of EU Risk Manage-

ment Plans. Within *chapter 2.1* these characteristics are compared between biologicals and small molecules. *Chapters 2.2 and 2.3* focus on the main characteristics of post-marketing identified safety issues for biologicals that necessitated a safety-related regulatory action. Among others the timing and nature of the safety problems was studied. In *chapter 2.2* all biologicals are addressed and compared whereas *chapter 2.3* focuses on orphan drugs. There a differentiation is made between biologicals and small molecule drugs.

Chapter 3 concentrates on spontaneously reported adverse drug reactions collected during use of biologicals in the clinical setting. In *chapter 3.1,* the safety profile of biologicals is studied and compared to the adverse drug reactions reported for the small molecule drugs. In addition, the safety profile is compared between different mechanistic classes of biologicals. *Chapter 3.2* evaluates a proposed classification system of adverse drug reactions of biologicals.

Chapter 4 concentrates on two studies in which risk factors and early markers are studied to identify patients at risk for a certain adverse drug reaction at an earlier stage. For these studies we use a cohort of rituximab users as a learning case. *Chapter 4.1* focuses on invasive Aspergillosis and *chapter 4.2* focuses on thrombocytopenia. Within *chapter 4.2* haematological markers and clinical characteristics are also used to evaluate the underlying mechanism of rituximab-induced thrombocytopenia.

Finally, in **chapter 5** the studies in this thesis are placed in a broader perspective and challenges in pharmacoepidemiological studies of biologicals are discussed. This chapter also provides recommendations for regulatory and clinical practice and for future research.

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Chapter 1

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Chapter 2

Regulatory actions and activities in the risk management of biologicals

Chapter 2.1

Evaluation of post-authorisation safety studies in the first cohort of EU risk management plans at time of regulatory approval

Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Sabine M.J.M. Straus
Toine C.G. Egberts
Stella Blackburn
Ingemar Persson
Hubert G.M. Leufkens

Chapter 2.1

Abstract

Background: Since November 2005, an EU Risk Management Plan (EU-RMP) has had to be submitted as part of a marketing application for all new chemical entities in the EU. In the EU-RMP, the safety profile of the medicine has to be described and pharmacovigilance activities should be proposed to study further safety concerns during use of the drug in the real-world setting. These activities include, for example, collection of spontaneously reported adverse events and post-authorisation safety studies (PASS). Since the submission of an EU-RMP is a relatively new requirement, there is limited knowledge on the quality and completeness of the study protocols of PASS at the time of approval and there are no data on the influence of certain drug characteristics on the proposed pharmacovigilance activities.

Objective: To examine the types of proposed pharmacovigilance activities in a sample of EU-RMPs, describe and evaluate the methodology of PASS, identify problems and propose remedies, and compare characteristics between biologicals and small molecules.

Methods: Eighteen EU-RMPs (nine for biologicals, nine for small molecules) given a positive decision regarding the marketing application by the Committee for Medicinal Products for Human Use between November 2005 and May 2007 were included in this descriptive cohort study. The EU-RMPs were selected over time and different therapeutic areas. Classification of the safety concerns ('important identified risks', 'important potential risks', 'important missing information' within the EU-RMP was studied. For PASS, data source (registry, population-based database, sponsor-owned clinical trial database), source of study population to be included in PASS and comprehensiveness of study protocol (full protocol, limited protocol, study synopsis, short description, commitment without further information) were studied.

Results: Compared to small molecules, safety concerns for biologicals were less frequently classified as important identified risks (relative risk [RR] 0.6; 95% CI 0.3-1.0) and more frequently as important missing information (RR 1.6; 95% CI 1.0-2.7).
Forty-seven PASS were proposed; 31 for biologicals and 16 for small molecules. Compared with studies proposed in population-based databases (4 for biologicals, 8 for small molecules), studies in registries (18 for biologicals, 4 for small molecules) were more frequently proposed for biologicals than for small molecules (RR 2.5; 95% CI 1.1- 5.7). About 60% of the proposed PASS will include EU inhabitants. No full study protocols were submitted; 26% involved a limited study protocol, 33% a study synopsis, 37% a short description and 4% a commitment without further information.

Conclusion: Approximately 40% of the study proposals for PASS were classified as a short description or a commitment to perform a study without further information precluding an adequate scientific assessment. Studying non-EU populations may give rise to difficulties with generalisability of the results to the EU due to differences in patient characteristics, differences in the indication for the medicine and different healthcare systems. This study emphasises the need for more complete study proposals to be submitted earlier on in the evaluation period and for the inclusion of EU inhabitants in PASS. In addition, differences in the characteristics between biologicals and small molecules, e.g. in the data source proposed, support the need for individualised tailored PASS depending on the type of drug.

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Background

The first spontaneous reports suggesting an association between the use of tumour necrosis factor antagonists and the occurrence of tuberculosis occurred during use of the drug in the post-marketing setting; the occurrence of tuberculosis had not been identified in pre-approval randomised clinical trials.[1,2] A recent study has shown that approximately one out of four biologicals approved in the US and/or the EU required a safety-related regulatory action, defined as written communications to healthcare professionals and black-box warnings, after approval of the drug by the regulatory authorities.[3] This illustrates the need for safety data to be gathered throughout the life cycle of a medicine due to the known limitations of clinical trials in predicting safety in 'real-world' use.[4] Therefore, post-marketing data offer a valuable and necessary complement to pre-registration studies in continuously evaluating the benefit-risk balance of marketed drugs, especially with respect to safety.[5] A more proactive approach towards the identification and quantification of safety concerns after marketing was aimed for in the International Conference on Harmonisation (ICH) guideline on pharmacovigilance planning, which was recommended for adoption in the three ICH regions (EU, Japan and the US) in November 2004.[6] The ICH guideline on pharmacovigilance planning was adopted in the EU, including additional requirements, in November 2005 by the obligatory submission of an EU Risk Management Plan (EU-RMP) as part of the marketing application of innovative medicines (Table 1). In the EU-RMP, the safety profile of the medicine has to be described and pharmacovigilance activities should be proposed to study further safety concerns, i.e. the important identified and/or important potential risks and important missing information. What constitutes an important identified risk, an important potential risk or important missing information is defined as a risk that could impact the benefit-risk balance of the product or have implications for public health. The proposed pharmacovigilance activities can include spontaneous reporting, post-authorisation safety studies (PASS) and clinical trials. The formalisation of the post-authorisation development plan for a medicine based on proactive pharmacovigilance is a new legal based tool[7-9] and at the moment

Table 1: Situations when an EU-Risk Management Plan is needed for the marketing application in Europe.[7,8]

- With the application for a new marketing authorization for:
   new chemical entities
   biosimilars
   generic/hybrid medicinal product where a safety concern requiring additional risk
   minimisation activities has been identified with the reference medicinal product
- With an application involving a significant change in marketing authorization (e.g. new dosage form, new route of administration or new manufacturing process for a biotechnologically-derived product) unless it has been agreed that submission is not required
- On request from a competent authority
- On the initiative of the marketing authorization applicant/marketing authorization holder

there is limited knowledge on the quality and completeness of the study protocols of PASS at the time of approval.

Therefore, the primary objective of the present study, as part of a quality review of EU-RMPs at the European Medicines Agency (EMEA), is to examine the type of pharma-covigilance activities in a sample of EU-RMPs, describe and evaluate the methodology of PASS as presented in the EU-RMPs at the time a positive decision regarding the marketing application is made by the Committee for Medicinal Products for Human Use (CHMP), identify problems with PASS and propose remedies.

To assess differences in the characteristics of EU-RMPs and PASS between different types of drugs, the drugs were classified into biologicals and small molecules. Biologicals and small molecules have different characteristics and, therefore, differ with respect to potential drug hazards. Potential drug hazards for biologicals include the complex pro-duction and purification process compared with small molecules, which are synthesised chemically; the high potential for the formation of antibodies, which is low for small mol-ecules; and the limited predictability of preclinical studies to identify clinical conse-quences.[10-15] The major implications of changes to the production process of biologicals can be clearly illustrated by the increased incidence of pure red-cell aplasia in patients treated with a particular formulation of recombinant human epoetin (rHuEPO) in which human serum albumin was replaced by polysorbate 80 and glycine.[11,12] The change in product formulation increased the immunogenicity of the particular rHuEPO, which caused neutralising antibodies, not only against rHuEPO but also against the endogenous erythropoietin.[11-14,16] The limited predictability of preclinical data for biologicals can be illustrated by the TeGenero trial in which healthy volunteers treated with the superagonist anti-CD28 monoclonal antibody TGN1412 developed a severe cytokine storm, which had not been predicted from preclinical trial data.[17] In addition, compared with small mol-ecules, biologicals are often used in a specialised (hospital) setting so large population-based databases (which often mainly include general practitioner and public pharmacy data) are likely to contain limited or no information on biologicals. These differences may affect proposals for EU-RMPs and PASS; therefore, the secondary objective of the present study is to compare the type of pharmacovigilance activities and the methodology of PASS between biologicals and small molecules.

Methods

The EU-RMP

An EU-RMP (Table 1) consists of two parts. The first part consists of the safety specifi-cation and the pharmacovigilance plan. The safety specification aims to provide an overview of the results and possible limitations of the pre-registration studies, whilst the pharmacovigilance plan describes the proposed pharmacovigilance activities to further study the safety concerns. At the end of the safety specification, a summary of the im-portant safety concerns is provided and this list is the basis for what needs to be discussed in the pharmacovigilance plan and the second part of the EU-RMP. A safety concern is defined in the Guideline on Risk Management Systems[8] as an 'important identified risk', 'important potential risk' or 'important missing information'. Safety concerns may be

based on the expected safety concerns related to the characteristics of the drug or the unexpected safety concerns, not predictable by the characteristics of the drug but identified in the pre-registration studies or during the post-authorisation activities. For each safety concern, pharmacovigilance activities encompassing both routine, e.g. collection of spontaneous reports of suspected adverse drug reactions, and additional activities, e.g. PASS, should be discussed. The second part of the EU-RMP consists of an evaluation of the need for risk minimisation activities, and, if considered necessary, a risk minimisation plan should also be provided.[7,9,18] The Guideline on Risk Management Systems for Medicinal Products for Human Use can be found in Volume 9A of The Rules Governing Medicinal Products in the European Union.[7] The template for EU-RMPs is described in annex C of this guideline.[19]

In the period November 2005 to May 2007, a total of 59 EU-RMPs (36 EU-RMPs for small molecules and 23 EU-RMPs for biologicals) were submitted as part of a new marketing application for centrally authorised products in Europe (not including line extensions). Of these, 18 EU-RMPs (nine biologicals [as defined by the EMEA[16]] and nine small molecules) were sampled and included in this descriptive cohort study. The sample of 18 EU-RMPs was selected over time and different therapeutic areas to obtain a comprehensive overview of EU-RMP practice. The EU-RMP or study protocol submitted in the final EU-RMP being part of the positive decision by the CHMP was included in the analysis. Information was obtained from EU-RMPs provided by the EMEA and from the European Public Assessment Reports, accessible via the EMEA web site [www.emea.europa.eu].

Safety concerns and types of pharmacovigilance activities to address them

Safety concerns were classified according to the EU Guideline on Risk Management Systems for Medicinal Products for Human Use[8] as important *identified* risks (adequate evidence of association with the medicinal product), important *potential* risks (there is a basis for suspicion of an association with the medicinal product but the association has not been confirmed) or important *missing information*, including populations not studied in the pre-authorisation phase, which may form part of the target population post-authorisation. 'Important' in this context means an identified risk, potential risk or missing information that could impact on the benefit-risk balance of the product or have implications for public health.[7]

The activities proposed to study safety concerns were classified as 1) routine pharmacovigilance; 2) PASS; 3) clinical trials; and 4) others. Routine pharmacovigilance, PASS and clinical trials were defined as laid down in Volume 9A of *The Rules Governing Medicinal Products in the European Union*.[7]

1) *Routine pharmacovigilance:* Pharmacovigilance activities that should be conducted for all medicinal products and include the submission of Periodic Safety Update Reports and reporting of spontaneous adverse events.

2) *PASS:* A pharmacoepidemiological study (non-interventional study) or a clinical trial (interventional study) carried out in accordance with the terms of the marketing authorisation, conducted with the aim of identifying or quantifying a safety hazard

relating to an authorised medicinal product.

3) *Clinical trials:* any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of one or more investigational medicinal product(s) and/or to identify any adverse reactions to one or more investigational medicinal product(s) and/or to study absorption, distribution, metabolism and excretion of one or more investigational medicinal products with the objective of ascertaining its (their) safety and/or efficacy. An investigational medicinal product is a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorisation but used or assembled (formulated or packaged) in a way that is different from the authorised form, or when used for an authorised indication or to gain further information about the authorised form.[7] Clinical trials that were specifically designed for identifying or quantifying a safety hazard relating to an authorised medicinal product were classified as PASS (2), and other clinical trials were classified as a clinical trial (3).

4) *Others:* Activities that could not be classified in one of the three previously described subgroups were classified as 'others' (4). An example of activities included in this group was a commitment to validate a new assay for antibodies.

Nature of safety concerns to be addressed by post-authorisation safety studies (PASS)

The nature of the safety concerns laid down in the safety specification to be addressed by PASS was classified at the System Organ Class (SOC) level according to the Medical Dictionary for Regulatory Activities (MedDRA), version 9.1. Special patient groups, for example children and hepatically impaired patients, are not included in MedDRA and were therefore classified as 'Special patient groups'. This was also done for safety concerns relating to the potential for off-label use, which was classified as 'Potential for off-label use'. Safety concerns that could not be classified according to MedDRA or the additional classification were classified as 'Others' and included, for example, 'Duration of protection and the need for a booster dose'.

Where several safety concerns belonging to different SOCs were included as one safety concern, both SOCs were counted; for example, misuse and abuse was classified in the SOCs 'Injury, poisoning and procedural complications' and 'Social circumstances', respectively.

PASS methodology

The study protocols of the proposed PASS were evaluated by two assessors (TJG and AKM-T) to obtain information on study type, study design and data source (including target population).

Study types

Study types were classified as comparative studies, non-comparative studies, background incidence studies and drug utilisation studies. The primary aim of comparative (drug under study vs. comparator) and non-comparative (no comparator group) studies was to

evaluate safety. Both study types were therefore classified as safety studies. Background incidence studies were studies to investigate the background incidence of certain adverse events of interest in the target population and drug utilisation studies evaluate how a medicinal product is marketed, prescribed and used in a population and how these factors influence the outcomes.[7] The classification of PASS was based on the provided primary objective of the PASS. PASS with more than one primary objective involving multiple study types were classified in all study types involved.

Study designs

Study designs were classified as cohort studies (prospective or retrospective), case-control studies, cross-sectional studies, (extensions of) randomized clinical trials (RCTs)**,** including large simple trials, and unknown. The (extensions of) RCTs are clinical trials that will be conducted after marketing of the drug, and can be classified as PASS, as previously described.

Data sources and target population

Data sources were classified as large, population-based databases, registries or sponsor-owned clinical trial databases. Registries were defined as data sources that include patients with a certain disease, condition or exposure and from which the data will be used for observational studies. Sponsor-owned clinical trial databases were defined as post-marketing (extensions of) clinical trials set up by the Marketing Authorization Holder. Information on the country/countries in which the study will be conducted was also collected. Registries were further categorized into (i) existing or newly set up registries; and (ii) ownership – registries owned by the pharmaceutical company or registries owned by other organizations, e.g. academia.

Comprehensiveness of PASS study protocols

Study protocols were classified as full study protocol, limited study protocol, study synopsis, (very) short description or a commitment (to perform the study) without further information. Study protocols were assessed based on the topics laid down in the Guideline for Good Pharmacoepidemiology Practice[20] and include objectives, study design, strategy and reasons for proposed design, study population, inclusion criteria, exclusion criteria, data source, health outcomes, potential confounders and effect modifiers, clear definition of health outcomes, exposure, selection criteria, comparison groups, study power, data analysis, description of quality assurance and quality control procedures, and limitations of the study.[20] A full study protocol contained 16 or 17 of the 17 topics; a limited study protocol contained between 11 and 15 of the 17 topics; a study synopsis contained between 6 and 10 topics; a (very) short description contained between 1 and 5 topics; and a commitment without further information did not contain any of the described topics.

Data analysis

Proportions and relative risks (RR) with corresponding 95% confidence intervals (CI) were calculated to compare the classification of safety concerns and studies relating to biologicals and small molecules. SPSS version 14.0 was used (SPSS Inc, Chicago, Ill).

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Table 2: Characteristics of the drugs included [www.emea.europa.eu].

Drug	Active substance	Indication	Date positive decision CHMP
Biologicals			
Atryn®	Antithrombin alfa	Prophylaxis of venous thromboembolism in surgery of patients with congenital anti-thrombin deficiency	1 June 2006
Elaprase®	Idursulfase	Long-term treatment of patients with Hunter syndrome	18 October 2006
Gardasil®	Human papilloma-virus vaccine (types 6, 11, 16, 18)	Prevention of high-grade cervical dysplasia (CIN 2/3), cervical carcinoma, high-grade vulvar dysplastic lesions (VIN 2/3) and external genital warts (condyloma acuminata) causally related to human papillomavirus types 6, 11, 16 and 18	27 July 2006
Lucentis®	Ranibizumab	Neovascular (wet) age-related macular degeneration	16 November 2006
Myozyme®	Recombinant human alglucosidase α	Long-term enzyme replacement therapy in patients with a confirmed diagnosis of Pompe disease (acid α glycosidase deficiency)	26 January 2006
Orencia®	Abatacept	In combination with methotrexate, indicated for the treatment of moderate to severe active rheumatoid arthritis in adult patients who have had an insufficient response or intolerance to other disease modifying anti rheumatic drugs, including at least one tumour necrosis factor inhibitor	19 March 2007
Preotact®	Parathyroid hormone	Osteoporosis in postmenopausal women at high risk of fractures	23 February 2006
Soliris®	Eculizumab	Paroxysmal nocturnal haemoglobinuria	27 April 2007
Tysabri®	Natalizumab	Single disease-modifying therapy in highly active relapsing-remitting multiple sclerosis for the following patient groups: patients with high disease activity despite treatment with a β- interferon; or patients with rapidly evolving severe relapsing-remitting multiple sclerosis	27 April 2006

Small molecules

Acomplia®	Rimonabant	As an adjunct to diet and exercise for the treatment of obese patients (BMI $\geq$30 kg/m^2) or overweight patients (BMI >27 kg/m^2) with associated risk factor(s), such as type 2 diabetes or dyslipidaemia	27 April 2006
Baraclude®	Entecavir	Chronic hepatitis B virus infection in adults with compensated liver disease and evidence of active viral replication, persistently elevated serum ALT levels and histological evidence of active inflammation and/or fibrosis	26 April 2006
Champix®	Varenicline	Smoking cessation in adults	27 July 2006
Circadin®	Melatonin	As monotherapy for the short-term treatment of primary insomnia characterized by poor quality of sleep in patients aged 55 years or over	27 April 2007
Competact®	Pioglitazone/ metformin	Type 2 diabetes mellitus patients, particularly overweight patients, who are unable to achieve sufficient glycaemic control at their maximally tolerated dose of oral metformin alone	1 June 2006
Invega®	Paliperidone	Schizophrenia	27 April 2007
Januvia®	Sitagliptin	Patients with type 2 diabetes mellitus to improve glycaemic control in combination with metformin when diet and exercise, plus metformin, do not provide adequate glycaemic control. For patients with type 2 diabetes in whom use of a PPAR agonist (i.e. a thiazolidinedione) is appropriate, Januvia® is indicated in combination with the PPAR agonist when diet and exercise plus the PPAR agonist alone do not provide adequate glycaemic control	24 January 2007
Sprycel®	Dasatinib	Adults with chronic-, accelerated- or blast- phase CML with resistance or intolerance to prior therapy, including imatinib and for adults with Philadelphia chromosome positive acute lymphoblastic leukaemia and lymphoid blast CML with resistance or intolerance to prior therapy	21 September 2006
Tygacil®	Tigecycline	Complicated skin and soft tissue infections; complicated intra-abdominal infections	23 February 2006

BMI = body mass index; CHMP = Committee for Medicinal Products; CIN = cervical intraepithelial neoplasia; CML = chronic myeloid leukaemia; PPAR = peroxisome proliferator-activated receptor; VIN = vulvar intraepithelial neoplasia

Results

The characteristics of the 18 products included (nine biologicals and nine small mole-cules) and the description of the included EU-RMPs are shown in Table 2 and Figure 1, respectively.

Figure 1: Inclusion and characteristics of the EU-Risk Management Plans (EU-RMPs).
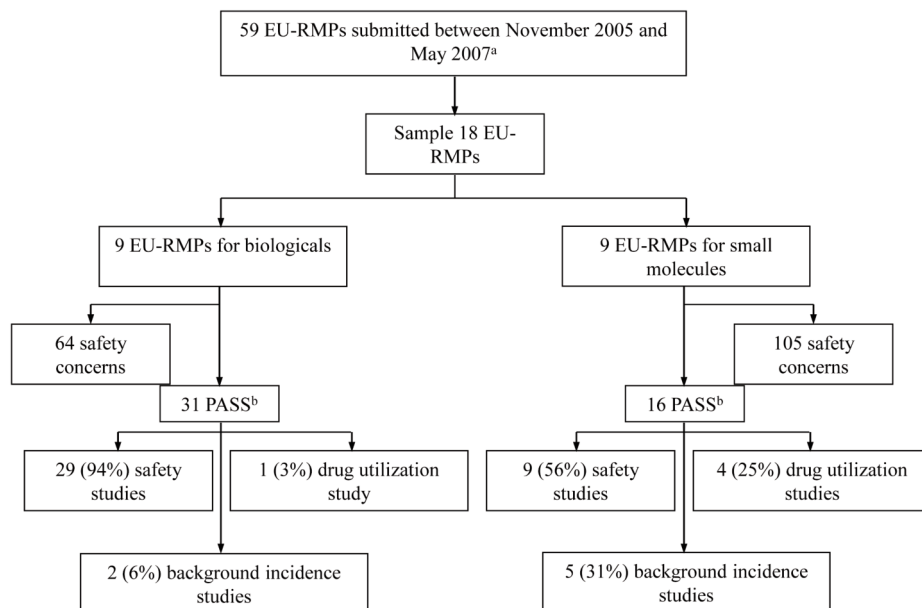
a) These included 36 EU-RMPs for small molecules and 23 EU-RMPs for biologicals.
b) The total number of study types proposed exceeds 47 because of multiple study types proposed in one study.

Classification of safety concerns and pharmacovigilance activities to address them

A total of 169 safety concerns were included in the 18 safety specifications. These safety concerns consisted of 50 (29.6%) important identified risks, 73 (43.2%) important po-tential risks and 46 (27.2%) important missing information (Table 3). For biologicals, as compared to small molecules, safety concerns were less frequently classified as important identified risks (RR 0.6; 95% CI 0.3-1.0) and more frequently as important missing in-formation (RR 1.6; 95% CI 1.0-2.7). Comparison of the important potential risks did not show a difference (RR 1.0; 95% CI 0.7-1.5) between biologicals and small molecules. Safety concerns were more frequently classified as either an important potential risk or important missing information (RR 1.2; 95% CI 1.0-1.5) for biologicals when compared with small molecules.

Routine pharmacovigilance was proposed to address more than 80% of all safety concerns. The major difference between biologicals and small molecules in pharmacovigilance activities to address safety concerns was the number of PASS and clinical trials proposed (Table 3).

Table 3: Safety concerns and how these are intended to be addressed [n (%)].

Safety concern	Safety concerns	Addressed by routine pharmaco-vigilance	Addressed by post-authorisation safety studies	Addressed by clinical trials	Others
Biologicals	64	52 (81)	52 (81)	16 (25)	2 (3)
Important identified risks	13 (20)	12 (92)	11 (85)	4 (31)	1 (8)
Important potential risks	28 (44)	21 (75)	21 (75)	9 (32)	1 (4)
Important missing information	23 (36)	19 (83)	20 (87)	3 (13)	0 (0)
Small molecules	105	89 (85)	14 (13)	53 (50)	6 (6)
Important identified risks	37 (35)	33 (89)	3 (8)	20 (54)	0 (0)
Important potential risks	45 (43)	37 (82)	6 (13)	18 (40)	4 (9)
Important missing information	23 (22)	19 (83)	5 (22)	15 (65)	2 (9)

Safety concerns addressed by proposed PASS

PASS were frequently proposed to study 'Special patient groups' and 'General disorders and administration site conditions' (Table 4). In addition, PASS were frequently proposed for safety concerns related to the SOCs 'Investigations', 'Infections and infestations', and 'Immune system disorders' for biologicals, and 'Psychiatric disorders' and 'Neoplasms, benign, malignant and unspecified (including cysts and polyps)' for small molecules.

PASS methodology

In the sample of 18 EU-RMPs, a total of 47 PASS were proposed – 31 for biologicals and 16 for small molecules (Figure 1).

Study types

Comparison of the number of safety studies (29 for biologicals vs. 9 for small molecules) with the number of background incidence and drug utilisation studies (3 for biologicals vs. 9 for small molecules) (Figure 1) showed that PASS were more frequently classified as safety studies for biologicals compared with small molecules (RR 1.7; 95% CI 1.1-2.6).

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Table 4: Classification of the most frequent reported safety concerns, by System Organ Class (SOC) [n (%)].

SOC	Safety concerns PASS biologicals (n = 52[a])	Safety concerns total biologicals (n = 64[a])	Safety concerns PASS small molecules (n = 14[a])	Safety concerns total small molecules (n = 105[a])
Special patient groups	15 (28.8)	18 (28.1)	4 (28.6)	24 (22.9)
General disorders and administration site conditions	6 (11.5)	8 (12.5)	1 (7.1)	8 (7.6)
Investigations	6 (11.5)	8 (12.5)	0 (0)	7 (6.7)
Infections and infestations	5 (9.6)	6 (9.4)	1 (7.1)	5 (4.8)
Immune system disorders	4 (7.7)	5 (7.8)	0 (0)	2 (1.9)
Nervous system disorders	0 (0)	1 (1.6)	0 (0)	11 (10.5)
Neoplasms, benign, malignant and unspecified (including cysts and polyps)	3 (5.8)	3 (4.7)	2 (14.3)	5 (4.8)
Psychiatric disorders	0 (0)	0 (0)	4 (28.6)	6 (5.7)
Cardiac disorders	1 (1.9)	1 (1.6)	1 (7.1)	4 (3.8)
Injury, poisoning and procedural complications	4 (7.7)	6 (9.4)	0 (0)	4 (3.8)
Others	10 (19.2)	10 (15.6)	3 (21.4)	35 (33.3)

a) Sum of the columns exceeds the total number of safety concerns due to safety concerns including multiple safety issues categorized in different SOCs.

PASS = post-authorisation safety studies

Study designs

The study designs proposed were cohort design (n = 35), nested case-control design (n = 5), RCT (n = 3), extension of RCT (n = 8) and in one case the study design could not be established. All (extensions of) RCTs were open-label studies. No cross-sectional studies were proposed. Of the 35 cohort studies, 24 were prospective, 10 retrospective and 1 study was not classifiable on the data given.

Data source and target population

The data source used differed between biologicals and small molecules. For biologicals, 18 (58%) of the studies were proposed in registries and four (13%) in large-population based databases. For small molecules, four (25%) of the studies were proposed in registries and eight (50%) in large-population based databases. A comparison of the number of studies proposed in registries with the number of studies proposed in large-population based databases showed that compared with small molecules, studies for biologicals are more often proposed in registries (RR 2.5; 95% CI 1.1-5.7). Nine (29%) of the studies proposed for biologicals and two (13%) of the studies proposed for small molecules will be conducted in sponsor-owned clinical trial databases. For two PASS proposed for small molecules, the data source was unknown. All cohort studies proposed in registries had a prospective nature.

Twelve (26%) of the 47 PASS proposed will be conducted in the EU population, 15 (33%) in the non-EU population (mainly US) and 17 (37%) in a multinational setting, including

the EU. For three studies (7%), this information could not be ascertained.

Further specification of the 22 studies proposed in registries showed that this involved 10 (45%) existing registries and 11 (50%) newly initiated registries. For one study (4.5%), this information could not be retrieved. The pharmaceutical industry owned 11 (50%) of the registries and other institutions owned 9 (41%) of the registries. For two registries (9%), this information could not be retrieved. In total, 10 of the 11 registries owned by the pharmaceutical industry were newly initiated and all nine registries owned by other organisations already existed.

Comprehensiveness of PASS study protocols

None of the 46 PASS (one background incidence study was excluded since this study was part of the EU-RMP but had already been conducted before licence approval) had a full study protocol at the time of a positive decision by the CHMP. A limited protocol was submitted for 12 (26%) of the 46 PASS, 15 (33%) had a study synopsis, 17 (37%) had a (very) short description and two (4%) had a commitment to perform a study without further information. Topics most frequently missing from the submitted study protocols were: strategy and reasons for proposed design (discussed in 2 protocols); limitations of the study (in 2 protocols); description of quality assurance and quality control procedures (in 4 protocols); potential confounders and effect modifiers (in 8 protocols); and clear defined health outcomes (in 11 protocols). Study protocols were generally more complete for biologicals than for small molecules, as shown by the fact that a limited study protocol or study synopsis (63% for biologicals vs. 50% for small molecules) seemed to be submitted more frequently than a (very) short description or a commitment to perform a study without further information (37% for biologicals vs. 50% for small molecules) (RR 1.3; 95% CI 0.7-2.2) (Figure 2).

Figure 2: Comprehensiveness of the study protocols submitted.

Legend:
- □ Limited study protocol
- ■ Study synopsis
- □ (Very) short description
- ▨ Commitment to perform a study without further information
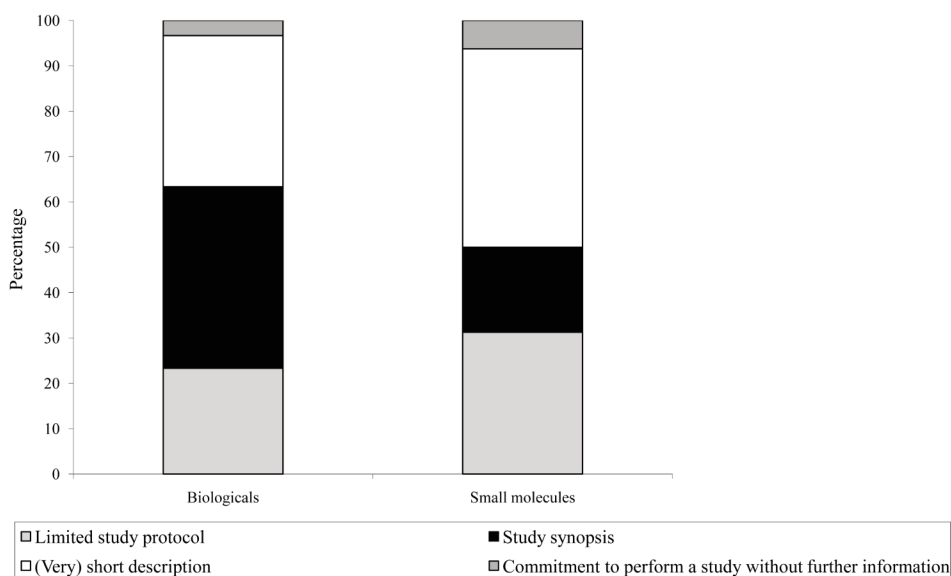
Discussion

This first review on the types of pharmacovigilance activities and the methodology of PASS in a sample of EU-RMPs provides valuable information on current EU-RMP practice and is important to help identify problems with EU-RMPs/ PASS at this early phase of EU-RMP practice, along with a focus on possible remedial actions. It was shown that more than 80% of the safety concerns will be addressed by routine pharmacovigilance, including spontaneous reporting of suspected adverse drug reactions. Spontaneous reporting has an important function in the detection, of new, rare and/or serious adverse events; however, certain limitations exist, including under-reporting and a difficult causality assessment.[15,21,22] 25% of the safety concerns for biologicals and 50% of the safety concerns for small molecules will be addressed by additional (extensions of) clinical trials**.** Extensions of clinical trials are a relatively uncomplicated method to follow patients over a longer period of time. However, limitations of clinical trials include a homogenous population and they are often underpowered to detect rare adverse events.[23]

PASS offer an important new tool to actively study safety concerns in the real-world setting. However, as shown by the present study, certain problems have been identified related to PASS, which need to be improved in the future. It was shown that no full study protocols had been submitted at the point of a positive decision, that 26% had submitted a limited study protocol, 33% a study synopsis and 37% a (very) short description. A commitment to perform a study without further information involved 4% of the PASS. The limited availability of full/limited study protocols and study synopses during the decision-making process is of major concern since this precludes a proper scientific assessment by the regulatory authorities. Although some protocols were requested to be provided post-authorisation, not having at least a study synopsis during decision making makes it impossible to assess the likelihood of the PASS providing the necessary safety information, which is counterintuitive to the spirit of proactive risk management practice. In addition, the lack of a protocol makes it impossible to assess the feasibility of the proposed PASS during decision making, which increases the risk that the required safety information will not be forthcoming. The Guideline on Risk Management Systems[8] states that additional pharmacovigilance activities should be designed and conducted according to the Guidelines for Good Pharmacoepidemiological Practice and that protocols (draft or otherwise) for any formal study should be provided. In addition, in Annex C to this guideline (the template for the EU-RMP) it is stated that full study protocols should be annexed to the EU-RMP; however, Annex C was adopted by the CHMP in September 2006 and was therefore not available for a large part of this study period.[7] Although requested in the guideline, it might be difficult for marketing applicants to submit study proposals early in the application cycle since regulators often request different approaches from that suggested by the applicant, ask for PASS late in the evaluation process or identify new safety concerns during the evaluation process when additional data are provided in the applicants' responses to questions. In addition, the proposed indication (and hence the target population) may change during the evaluation procedure.

Ideally, marketing authorisation applicants and regulators should have active discussions on PASS early in the evaluation process or prior to the first submission of the dossier to the regulatory authorities by way of scientific advice. If the submission of study protocols

is not feasible during the decision making process, clear timelines should be set by which full study protocols should be submitted as part of the post-approval commitments.

At the moment of a positive decision by the CHMP, the information on the safety profile of biologicals is more limited than that of small molecules, in part due to the specific characteristics of biologicals, e.g. the limited predictability of preclinical data to clinical data for biologicals.[10,14] This finding reinforces the need for more active pharmacovigilance of biologicals to obtain information on the potential risks and missing information. This was supported by the 31 PASS proposed for biologicals compared with the 16 PASS for small molecules and the significantly higher overall number of safety studies for biologicals. However, this might also be (partly) due to the fact that three of the biologicals included have an orphan designation compared with one small molecule. It is known that clinical trials for orphan drugs include fewer patients compared with drugs with no orphan designation,[24] resulting in limited knowledge on the safety profile. In addition, differences were found between biologicals and small molecules in the type of safety concerns, the type of PASS and the data source used. These findings support the need for individualised tailored PASS, depending on the type of product.

The studies will include EU inhabitants in about 60% of the proposed studies, and about one-third of the studies will include non-EU inhabitants, mainly from the US. Extrapolation of non-EU results to the European patient population may be affected by differences in patient characteristics, differences in the indication for the medicine and different healthcare systems. In addition, it might be that a drug, for which the EU-RMP proposes studies in the non-EU countries, has not yet been approved by the country in which the study is proposed. If the marketing application will not be granted in the non-EU countries, the post-marketing studies proposed will not be conducted.

In this study, reviewing the most recent study protocol submitted in the final EU-RMP, being part of the CHMP decision-making process, can be considered a limitation since study protocols can be amended after marketing by submission of a more extensive study protocol and/or availability of new safety data. Inclusion of study protocols submitted up to the date of a positive decision will, however, provide insight into the data on which the decision by the CHMP was based. In addition, this resulted in comparable information between drugs.

The EU-RMPs included for biologicals and small molecules were sampled over time (November 2005–May 2007) and different therapeutic areas. It can be debated whether the selected EU-RMPs are a good representation of the population of biologicals and small molecules (e.g. with regard to the indication of the drugs) and if there has been a change in the characteristics of EU-RMPs over time (improvement from learning and interventions). However, a closer look at the number of products approved in the different Anatomical Therapeutic Chemical (ATC) classes in relation to the number of products included in this study showed that for most of the ATC classes in which three or more products are approved, between 21% and 45% of the products are included in this study. This does not apply to the ATC classes 'Genito-Urinary System and Sex Hormones' and 'Various', in which three products were approved, of which none were included in this study. This might influence the generalisability of our study and can be considered a

limitation. However, in general it can be concluded that, based on the ATC classification, the sample of EU-RMPs included in this study is representative of the other EU-RMPs submitted as part of an initial marketing application. Although learning over time will improve the quality of EU-RMPs, the sampling has resulted in the inclusion of a range of different therapeutic areas over time, for which the results found show a good overview of current practice, which has a positive impact on the generalisability of the results found in this study. Finally, it should be noted that parts of the information used in this study are not available in the public domain.

The limited availability of full/limited study protocols and study synopses, available at the time of a positive decision by the CHMP, is of concern since this precludes a proper scientific assessment of the feasibility and value of the study. Discussion of the study protocols between the marketing authorization applicant and the regulatory authorities at an early stage in the application cycle is encouraged since this will facilitate assessment and might improve the quality and feasibility of the proposed studies. In some cases, the number of patients who will be treated is difficult to calculate, depending on, for example, reimbursement of the drug, indications approved in different countries (for non-EU studies) and uptake by clinicians in daily clinical practice. Although uncertainties will remain, adequate sample size calculations will provide important information on the power of the study and are therefore highly recommended. The potential problems encountered with the exclusion of EU inhabitants in about one-third of the PASS proposed should be taken into consideration, both by the pharmaceutical industry and the regulatory authorities, and pharmaceutical companies are encouraged to include EU inhabitants.

Large population-based databases and registries are an important tool for PASS. However, the marketing authorisation applicant is advised to clearly assess the validity of a data source. In addition, it would be very useful if the marketing authorisation applicant provided information as to the rationale of using the proposed data source. In addition, it should be emphasised that data from healthcare systems on exposed individuals should be accessible in order to build registries for clinical follow-up.

The results of this study will add to EMEA activities to improve the approval procedures, achieve a more timely and rapid regulatory review of the protocols of PASS, have an earlier interaction with the responsible company and to consult the proper expertise, e.g. in pharmacoepidemiology. The new initiative to facilitate networking between competence centres in pharmacoepidemiological research and for co-ordinating data resources in the EU might further improve proactive pharmacovigilance in the EU. This network is being developed under the lead of the EMEA (European Network of Centres for Pharmacoepidemiology and Pharmacovigilance and can be used in future by all relevant stakeholders involved [www.encepp.eu].[25]

In future, as part of the quality review of EU-RMPs at the EMEA, it will be relevant to follow-up the proposed PASS included within the present study, and to have a closer look at the status of the study and possible amendments to the study protocol at a later stage. In addition, it will be very interesting to study the effect of EU-RMPs on patient safety and the early identification of postmarketing safety problems.

Conclusions

This study showed that EU-RMP practice should be further improved by means of the submission of more complete study protocols at the moment of a positive decision by the CHMP, since at moment of regulatory approval 40% of the study proposals were classified as a short description or a commitment without further information, precluding an adequate scientific assessment. Problems might be expected based on the inclusion of non-EU inhabitants with regard to differences in patient characteristics, differences in the indication for the medicine and different healthcare systems. Inclusion of EU inhabitants in PASS is therefore highly recommended. In addition, differences in the characteristics between biologicals and small molecules, e.g. in the safety problems to be encountered and the data source proposed, support the need for individualized tailored PASS depending on the type of drug.

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Chapter 2.2

Safety-related regulatory actions for biologicals approved in the United States and the European Union

Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Sabine M.J.M. Straus
Huub Schellekens
Hubert G.M. Leufkens
Toine C.G. Egberts

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Abstract

Context: Biologicals are a relatively new class of medicines that carry specific risks (e.g., immunogenicity). However, limited information is available on the nature and timing of safety problems with their use that were identified after approval.

Objective: To determine the nature, frequency, and timing of safety-related regulatory actions for biologicals following approval in the United States and/or the European Union.

Design and Setting: Follow-up of a group of biologicals approved in the United States and/or European Union between January 1995 and June 2007. Vaccines, allergenic products, and products for further manufacture and transfusion purposes were excluded.

Main Outcome Measures: Nature, frequency, and timing of safety-related regulatory actions defined as 1) dear healthcare professional letters (United States) and direct healthcare professional communications (European Union), 2) black box warnings (United States), and 3) safety-related marketing withdrawals (United States and European Union) issued between January 1995 and June 2008.

Results: A total of 174 biologicals were approved (136 in the United States and 105 in the European Union, of which 67 were approved in both regions). Eighty-two safety-related regulatory actions (46 dear healthcare professional letters, 17 direct healthcare professional communications, 19 black box warnings, and no withdrawals) were issued for 41 of the 174 different biologicals (23.6%). The probability of a first safety-related regulatory action, derived from Kaplan-Meier analyses, was 14% (95% confidence interval [CI] 9%-19%) 3 years after approval and 29% (95% CI 20%-37%) 10 years after approval. Biologicals first in class to obtain approval had a higher risk for a first safety-related regulatory action compared with later approved products in that class (12.0/1000 vs. 2.9/1000 months, respectively; hazard ratio, 3.7 [95% CI 1.5-9.5]).

Warnings mostly concerned the classes general disorders and administration site conditions, infections and infestations, immune system disorders and neoplasms benign, malignant, and unspecified.

Conclusions: The nature of safety problems identified after approval for biologicals is often related to the immunomodulatory effect (infections). Because the biologicals first to be approved in a class were more likely to be subjected to regulatory action, close monitoring is recommended.

Background

Biologicals, defined as products of which the active substance is produced by or extracted from a biological source, represent an important and growing part of the therapeutic arsenal.[1] In the United States, the first biological, recombinant insulin, was approved in October 1982.[2] Since then, more than 250 biologicals, including recombinant (blood) products, monoclonal antibody–based products, and recombinant vaccines have been approved by regulatory authorities.[3] Between 2003 and 2006, biologicals represented 24% and 22% of all new chemical entities approved by US and EU regulatory authorities, respectively.[4] Sales of biotech products in the United States showed an annual growth rate of 20% between 2001 and 2006 compared with 6% to 8% in the pharmaceutical market.[5]

Knowledge of a new drug is incomplete at the time of approval, especially with reference to its safety profile, due to a variety of factors including constraints in the sample size and the design of randomised controlled trials.[6,7] Although this also applies to small molecules, biologicals carry specific risks. In contrast to small molecules, which are synthesised chemically, biologicals are derived from living sources (e.g., humans, animals, cells, and micro-organisms). The production and purification process of biologicals is more complex, involving numerous steps with the risk of influencing the characteristics of the end product at any single step in the production cascade.[8,9] Small differences and changes in the production process can therefore have major implications on the safety profile of biologicals. For example, the incidence of pure red cell aplasia in patients treated with recombinant human epoetin, an extremely rare complication induced by antibodies, was elevated in patients taking one particular formulation of recombinant human epoetin in which human serum albumin was replaced with polysorbate 80 and glycine.[10,11] However, the exact mechanism underlying the increased risk of pure red cell aplasia after the formulation change is not yet fully understood.[12] The risk of contamination with pathogens by the donor is another problem related to the production process (e.g., for products extracted from human blood or plasma).[13]

Biologicals are specifically prone to the induction of immunogenicity. In many cases, the consequence of immunogenicity is not clinically relevant. However, in some cases immunogenicity can lead to loss of efficacy of the drug or, even worse, lead to autoimmunity to endogenous molecules. There can be a major clinical impact if a natural protein with essential biological activity is neutralised by antibody formation.[8,10,14,15]

The predictability of preclinical data to humans is limited for biologicals due to the species-specific action and immunogenic properties in animals.[9] A recent striking example of this has been the occurrence of serious adverse events in healthy volunteers participating in a phase 1 clinical study of TeGenero's TGN1412, a CD28 agonist monoclonal antibody. The observed cytokine storm following infusion had not been observed in the preclinical studies of TGN1412.[16] To obtain valuable results from the preclinical (toxicology) studies, a relevant test animal should not only be selected based on pharmacological activity and low immunogenicity, but suitable pharmacokinetic properties also should be taken into account.[17,18] In some cases, the preclinical program is further complicated by a complex pharmacodynamic-pharmacokinetic relationship that can be illustrated by the delayed pharmacodynamic effect of peginterferon interleukin 2, which

becomes apparent long after the drug has disappeared from the blood compartment. Another complicating factor is the occurrence of bell-shaped response curves often seen in animals, especially with cytokines, in which the desired effect disappears after an increase of the dose.[19] Biologicals mainly act extracellularly and toxicity is often attributed to an exaggerated pharmacology,[18] which can be illustrated by the occurrence of serious infections due to the immunomodulatory function of many biologicals (e.g., monoclonal antibodies and interferons).[20-22]

As shown in previous studies, the use of drugs in the post-approval real-world setting can lead to the identification of important safety problems, which may even result in the withdrawal of drugs from the market.[23,24] Because biologicals carry specific risks, and limited information is available on the nature and timing of safety-related regulatory actions issued after approval for biologicals, our study examines the nature of the safety-related regulatory actions issued for biologicals and determines the probability of a safety-related regulatory action being issued after approval. Based on the immunomodulatory function of many biologicals, it is expected that an important part of the warnings for biologicals are related to this characteristic. In addition, within the group of biologicals, differences in the risk of safety-related regulatory actions (e.g., between mechanistic classes) also are studied.

Methods

Biologicals

This study included biological medicinal products (biologicals) approved in the United States and/or the European Union between January 1995 and June 2007. Biologicals were defined according to the European Medicines Agency (EMEA).1 The same active substances marketed by different pharmaceutical companies and biosimilars were included in the study as separate biologicals.

For the United States, the study included biologicals approved by the Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research (CDER; data available from January 1996 onward). The information was obtained from the web sites of CBER [http://www.fda.gov/cber] and CDER [http:// www.accessdata.fda.gov/scripts/cder/drugsatfda], respectively. Biologicals licensed under the same application number were included only once. Within the European Union, biologicals granted a marketing authorisation were identified from the European public assessment reports for authorised medicinal products for human use. All information was retrieved from the web site of the EMEA [http://www.emea.europa.eu].

Biologicals with an extension of indication during the study period, vaccines, allergenic products (allergen patch tests and allergenic extracts), biological products for further manufacture, and biological products for transfusion purposes and maintenance of circulating blood volume were excluded.

Biologicals were classified in therapeutic classes according to the Anatomical Therapeutic Chemical classification system [http://www.whocc.no/atcddd], and were classified in the mechanistic classes of antibodies (including monoclonal antibodies), cytokines, enzymes, growth factors, hormones, interferons, receptors, and others/various. Monoclonal anti-

bodies were further classified into murine, chimeric, and humanised.[25,26]

Safety-related regulatory actions

Safety-related regulatory actions were defined as 1) written communications to health care professionals (dear healthcare professional letters [DHPLs] in the United States and direct healthcare professional communications [DHPCs] in the European Union), 2) post-approval black box warnings (United States only), and 3) market withdrawals due to safety reasons (United States and European Union). Safety-related regulatory actions were collected between January 1995 and June 2008, which ensured at least 1 year of follow-up for each of the biologicals being studied.

Dear healthcare professional letters were identified from MEDWATCH from 1996 onward [http://www.fda.gov/medwatch]. Direct healthcare professional communications were identified from the web site of the Medicines Evaluation Board in the Netherlands [http://www.cbg-meb.nl] and the European public assessment reports of the EMEA. The date of the letter was used as the date of the safety-related regulatory action. Letters not including safety warnings and follow-up letters of previously issued letters containing no new safety information were excluded.

Post-approval black box warnings were identified from the labels available from the web sites of CDER, CBER, MEDWATCH, and the marketing authorisation holder and announcements posted on the MEDWATCH web site. The latest approved label of every biological available from CDER was searched for a black box warning. When a black box warning was identified from the latest approved label, previously approved labels were checked to identify the date the black box warning was added, which was cross-checked with the information from MEDWATCH. Labels that could not be retrieved from CDER were retrieved from CBER, MEDWATCH, and/or the marketing authorisation holder. The date of the black box warning stated on the web sites of CDER, CBER, or MEDWATCH was included in the analysis. When the exact date of the black box warning could not be identified, the latest possible date the black box warning was issued was included in the analysis. No label could be retrieved for 1 biological, and this biological was excluded from the analysis of black box warnings.

Drug withdrawals for safety reasons were identified from MEDWATCH, CDER, and the European public assessment reports listed on the EMEA web site. The date of the decision was used in the analysis.

Source and nature of the safety information

The source of the safety-related regulatory action described in the communications to health care professionals was collected and classified as post-approval reports (including both spontaneous reports as well as pharmacoepidemiological studies and registries), clinical trial data, a combination of post-approval reports and clinical trial data, others, or unknown. Because information on the source of the safety-related regulatory action is normally not included in a black box warning, these warnings were excluded from this part of the analysis, in which the source of the safety-related regulatory action was studied. The nature of the safety information was coded according to the Medical Dictionary for

Regulatory Authorities version 9.1. The primary reasons for the dissemination of the safety-related regulatory action were included in the analysis. Safety information was encoded using 5 levels: lower-level term, preferred term, higher-level term, high-level group term, and system organ class, but only the system organ class level was used in the analysis.

Data analysis

The number of biologicals classified at the Anatomical Therapeutic Chemical level in the United States and European Union were compared by the χ^2 test. The mean time to a safety-related regulatory action was calculated by summing the times between approval and a warning and dividing by the total number of safety-related regulatory actions issued. The incidence of safety-related regulatory actions was calculated as a simple proportion. Kaplan-Meier survival curves were used to estimate the probability of the occurrence of a safety-related regulatory action for the total group of biologicals (those approved in either the United States or European Union), and for the subgroups (those approved in the European Union and United States separately, depending on the safety-related regulatory action of interest). For biologicals that acquired multiple safety-related regulatory actions, only the first regulatory action was included in the analysis.

Hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) were calculated using the Cox proportional hazards model. The HRs were calculated to assess if the first biological approved in a (new) chemical, pharmacological, and therapeutic subgroup, as defined by the Anatomical Therapeutic Chemical classification system, had a higher risk of a safety-related regulatory action compared with biologicals approved at a later stage. To evaluate if experience with biologicals (those approved at a later point in time) might influence the number of safety-related regulatory actions, 2 time frames were constructed that included biologicals approved between January 1995 and June 2001 (6.5 years) and between July 2001 and June 2007 (6 years). The HRs also were calculated to compare the risk of safety-related regulatory actions issued for different mechanistic classes of biologicals as described previously. Hormones were used as the reference group because there is long-term, extensive experience with hormones within the group of biologicals, and hormones are often imitations of naturally occurring substances.[26] For the subgroups of biologicals approved in the United States and European Union, the time-to-event distributions of DHPLs issued in the United States and DHPCs issued in the European Union were compared by HRs, which included a variance adjustment to account for statistical independence.

For a subgroup of biologicals approved in both the United States and European Union, the nature and timing of the safety-related regulatory action were compared descriptively. When a safety-related regulatory action was issued for a biological not approved in the other region at the time of the regulatory action, this regulatory action was excluded. The timing of a safety-related regulatory warning was classified (1) in the United States first (European Union >2 months later), (2) in the European Union first (United States >2 months later), and (3) in both the United States and European Union within a period of 2 months.

All statistical analyses were conducted by using the statistical software package SPSS version 14 (SPSS Inc, Chicago, Illinois) and S-PLUS version 6.2 (Insightful Corp, Seattle,

Washington). All hypotheses were tested using 2-sided tests with an α level of 0.05. All analyses were unadjusted because the objective of this study was descriptive and not etiologic in nature.

Results

A total of 174 biological medicinal products obtained approval between January 1995 and June 2007; this comprised 136 biologicals approved in the United States and 105 in the European Union, of which 67 biologicals obtained approval in both regions during the study period (Table 1).

The differences between the number of approved biologicals in the United States and European Union was mostly explained by differences in the Anatomical Therapeutic Chemical classes of insulins and analogues ($p<0.001$), other antianemic preparations ($p=0.10$), anterior pituitary lobe hormones and analogues ($p=0.08$), and immunoglobulins ($p=0.01$).

Table 1: Biologicals approved between January 1995 and June 2007, classified by therapeutic class (ATC) (N=174).

SOC	No. (%) biologicals approved		
	United States (n=136)	European Union (n=105)	p-value
Alimentary tract and metabolism			
Insulins and analogues	10 (7.4)	24 (22.9)	<0.001
Other alimentary tract and metabolism products	6 (4.4)	7 (6.7)	0.44
Blood and blood forming organs			
Antithrombotic agents	13 (9.6)	9 (8.6)	0.79
Vitamin K and other hemostatics	12 (8.8)	9 (8.6)	0.95
Other antianemic preparations	1 (0.7)	4 (3.8)	0.10
Other hematological agents	4 (2.9)	0	NA
Cardiovascular system	1 (0.7)	0	NA
Dermatologicals	1 (0.7)	1 (1.0)	0.85
Genito urinary systems and sex hormones			
Gonadotrophins and other ovulation stimulants	7 (5.1)	4 (3.8)	0.62
Systemic hormonal preparations, excl. sex hormones and insulins			
Anterior pituitary lobe hormones and analogues	13 (9.6)	4 (3.8)	0.08
Pancreatic hormones	2 (1.5)	0	NA
Calcium homeostatis	2 (1.5)	2 (1.9)	0.79
Antiinfectives for systemic use			
Immunoglobulins	15 (11.0)	2 (1.9)	0.01
Antineoplastic and immunomodulating agents			
Cytokines and immunomodulators	9 (6.6)	12 (11.4)	0.19
Immunosuppressive agents	12 (8.8)	11 (10.5)	0.67
Other antineoplastic agents	8 (5.9)	5 (4.8)	0.70
Musculo-skeletal system	2 (1.5)	3 (2.9)	0.45
Respiratory system	3 (2.2)	1 (1.0)	0.45
Sensory organs	2 (1.5)	1 (1.0)	0.72
Various	13 (9.6)	6 (5.7)	0.27

NA = data not applicable.

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Table 2: Biologicals with a Dear Healthcare Professional Letter (DHPL) in the United States.

Class of biological	Active substance	Drug Name	Drug approval date	Warning	Time to DHPL in years
Antibodies	Adalimumab	Humira	December 31, 2002	Serious infections in combination with anakinra, hypersensitivity reactions, hematologic events	1.8
	Basiliximab	Simulect	May 12, 1998	Severe acute hypersensitivity reactions	2.4
	Bevacizumab	Avastin	February 26, 2004	Arterial thromboembolic events	0.5
				Reversible posterior leukoencephalopathy syndrome	2.5
				Tracheoesophageal fistula	3.1
	Cetuximab	Erbitux	February 12, 2004	Hypomagnesaemia, infusion reactions	1.6
	Daclizumab	Zenapax	December 10, 1997	Increased mortality in a study, hypersensitivity reactions	5.6
	Efalizumab	Raptiva	October 27, 2003	Immune-mediated hemolytic anemia, serious infections, thrombocytopenia	1.7
	Ibritumomab tiuxetan	Zevalin	February 19, 2002	Severe cutaneous and mucocutaneous reactions	3.6
	Infliximab	Remicade	August 24, 1998	Adverse events due to antibodies	0.2
				Tuberculosis and other opportunistic infections	3.1
				Worsening heart failure	3.2
				Malignancies (lymphoma)	6.1
				Hepatotoxicity	6.3
	Natalizumab	Tysabri	November 23, 2004	Progressive multifocal leukoencephalopathy	0.4
				Progressive multifocal leukoencephalopathy	1.6
				Hepatotoxicity	3.2
	Normal immunoglobulin	Venoglobulin	January 10, 1997	Renal dysfunction and/ or acute renal failure	1.8
	Palivizumab	Synagis	June 19, 1998	Anaphylaxis	4.4
	Ranibizumab	Lucentis	June 30, 2006	Stroke	0.6
	Intravenous RhoD immune globulin	WinRho SDF Liguid	March 21, 2005	Intravascular hemolysis in ITP patients, potential interference with blood glucose measurement	0.7
	Rituximab	Rituxan	November 26, 1997	Severe infusion related adverse events	1.0
				Hepatitis B reactivation with related fulminant hepatitis	6.6

Category	Active substance	Brand name	Date	Adverse event	Value
	Technetium fanolesomab	Neutrospec	July 2, 2004	Cardiopulmonary events	1.4
	Trastuzumab	Herceptin	September 25, 1998	Hypersensitivity reactions, infusion reactions, pulmonary events	1.6
				Cardio toxicity	6.9
Cytokines	Denileukin diftitox	Ontak	February 5, 1999	Visual loss	7.1
	Oprelvekin (Interleukin-11)	Neumega	November 25, 1997	Papilledema	3.7
Enzymes	Drotrecogin alfa	Xigris	November 21, 2001	Mortality in patients with single organ dysfunction and recent surgery	3.2
				Central nervous system bleeding	3.4
	Eptacog alfa	Novoseven	March 25, 1999	Thrombotic and thromboembolic adverse events	6.7
	Nesiritide	Natrecor	August 10, 2001	Increased 30 day mortality	3.7
	Streptokinase	Streptase	November 15, 1997	Adverse events during off-label use; hypotension, hypersensitivity reactions, apnea, bleeding	2.2
Growth factors	Beclapermin	Regranex	December 16, 1997	Increased risk of mortality secondary to malignancy	10.5
Hormones	Darbepoetin alfa	Aranesp	September 17, 2001	Thrombotic events, increased mortality, tumor growth potential	3.3
				Pure red cell aplasia and severe anemia associated with neutralizing antibodies	4.1
	Insulin human Inhalation powder	Exubera	January 27, 2006	Increased mortality in a trial	5.4
				Primary lung malignancies	2.2
	Somatropin	Genotropin	August 24, 1995	Increased mortality in a trial	2.2
				Fatalities	7.8
Interferons	Interferon beta-1a	Avonex	May 17, 1997	Antibody formation	5.8
				Hepatic injury	7.8
Other/ various	Danaparoid sodium	Orgaran	December 24, 1996	Epidural or spinal hematomas	1.0
Receptors	Alefacept	Amevive	January 30, 2003	Contraindication in patients with HIV. Alefacept might accelerate disease progression or increase complications of disease	2.7
	Etanercept	Enbrel	November 2, 1998	Serious infections including sepsis	0.5
				Central nervous system disorders, pancytopenia	1.9

Table 3: Biologicals With a Direct Healthcare Professional Communication (DHPC) in the European Union.

Class of biological	Active substance	Drug Name	Drug approval date	Warning	Time to DHPC in years
Antibodies	Alemtuzumab	MabCampath	July 6, 2001	Cases of death related to infections	6.6
	Bevacuzimab	Avastin	January 12, 2005	Tracheoesophageal fistula	2.3
	Infliximab	Remicade	August 13, 1999	Tuberculosis	1.4
				Worsening heart failure	2.2
				Infections including tuberculosis, contraindication: heart failure	2.5
				Hepatosplenic T-cellymphoma	6.8
	Rituximab	Mabthera	June 2, 1998	Cytokine release syndrome	0.5
				Progressive multifocal leukoencephalopathy	8.8
	Trastuzumab	Herceptin	August 28, 2000	Cardio toxicity in combination with anthracyclines and need for cardiac monitoring	1.7
Cytokines	Anakinra	Kineret	March 8, 2002	Serious infections and neutropenia in combination with etanercept	0.9
Enzymes	Lepirudin	Refludan	March 13, 1997	Fatal anaphylactic reactions	5.6
Growth factors	Dibotermin alfa	Inductos	November 9, 2002	Postoperative oedema at application site	1.9
				Implant site fluid collections	4.5
Hormones	Insulin human inhalation powder	Exubera	June 18, 2008	Primary lung carcinoma	2.4
Others/ various	Botulinum toxin	Neurobloc	March 14, 2001	Muscle weakness, dysphagia, aspiration	6.3
Receptors	Etanercept	Enbrel	February 3, 2000	Blood dyscrasia (pancytopenia, aplastic anemia)	0.7
				Serious infections and neutropenia in combination with kineret	3.0

Table 4. Biologicals with a Black Box Warning in the US.

Class of biological	Active substance	Drug Name	Drug approval date	Warning	Time to BBW in years
Antibodies	Cetuximab	Erbitux	February 12, 2004	Cardiopulmonary arrest	2.0
	Gemtuzumab ozogamicin	Mylotarg	May 17, 2000	Hypersensitivity reactions including anaphylaxis, infusion reactions, pulmonary events, hepatotoxicity	0.8
	Ibritumomab tiuxetan	Zevalin	Febraury 19, 2002	Severe cutaneous and mucocutaneous reactions	3.6
	Infliximab	Remicade	August 24, 1998	Risk of infections Hepatosplenic T-cell lymphomas	3.5 7.7
	Natalizumab	Tysabri	November 23, 2004	Progressive multifocal leukoencephalopathy	1.5
	Omalizumab	Xolair	June 20, 2003	Anaphylaxis	4.0
	Rituximab	Rituxan	November 26, 1997	Fatal infusion reactions, tumor lysis syndrome, severe mucocutaneous reactions Progressive multifocal leukoencephalopathy	8.2 9.2
	Trastuzumab	Herceptin	September 25, 1998	Infusion reactions, pulmonary toxicity	3.2
Cytokines	Oprelvekin (Interleukin-11)	Neumega	November 25, 1997	Allergic reactions including anaphylaxis	4.8
Enzymes	Laronidase	Aldurazyme	April 30, 2003	Life-threatening anaphylactic reactions	5.0
Growth factors	Beclapermin	Regranex	December 16, 1997	Increased risk of mortality secondary to malignancy	10.5
Hormones	Darbepoetin alfa	Aranesp	September 17, 2001	Increased mortality, cardiovascular events, thromboembolic events, tumor progression	5.5
Interferons	Interferon alfacon	Infergen	October 6, 1997	Fatal or life-threatening neuropsychiatric, auto-immune, ischemic and infectious disorders	5.1
	Peginterferon alfa-2a	Pegasys	October 16, 2002	Birth defects and/ or death of the fetus in combination with ribavirin	0.1
	Peginterferon alfa-2a	Pegintron	January 19, 2001	Birth defects and/ or death of the fetus in combination with ribavirin	0.5
Others/ various	Danaparoid sodium	Orgaran	December 24, 1996	Spinal/ epidural hematomas	1.1
Receptors	Etanercept	Enbrel	November 2, 1998	Risk of infections	9.4

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During the period under review, 82 safety-related regulatory actions were issued for 41 of the 174 biologicals (23.6%). These included 46 DHPLs (Table 2), 17 DHPCs (Table 3), and 19 black box warnings (Table 4).

No biologicals were withdrawn due to safety reasons. The mean time to elicit a safety-related regulatory action was 3.7 years and 70.7% of the safety-related regulatory actions were issued within 5 years after approval. The Kaplan-Meier probability of a biological requiring its first safety-related regulatory action was 14% (95% CI 9%-19%) 3 years after approval and 29% (95% CI 20%-37%) 10 years after approval.

Biologicals that were the first to be approved in their chemical, pharmacological, and therapeutic subgroup had a significantly higher risk for the occurrence of its first safety-related regulatory action compared with later approved products (HR 3.7; 95% CI 1.5-9.5). When the first approved products also included biologicals approved in a chemical, pharmacological, and therapeutic subgroup in which small molecules already had been approved, a significant increased risk for the first safety-related regulatory action was found as well (HR 2.3; 95% CI 1.1-4.8) (Table 5). Biologicals approved between July 2001 and June 2007 had a non-significantly higher risk for their first safety-related regulatory action compared with biologicals approved between January 1995 and June 2001 (HR 1.5; 95% CI 0.8-2.8) (Table 5).

Table 5: Risks for a first safety-related regulatory action.

	Number of biologicals	No. of events	Follow-up Mo	Incidence rate/ 1000 months	Hazard ratio (95%CI)
No small molecules previously approved in class[a]					
First biological	8	5	416.3	12.0	3.7 (1.5-9.5)
Later biologicals	162	35	11916.8	2.9	1 [reference]
Small molecules previously approved in class[a]					
First biological	19	9	1380.6	6.5	2.3 (1.1-4.8)
Later biologicals	151	31	10952.5	2.8	1 [reference]
Timing of approval					
January 1995 – June 2001	90	23	9007.3	2.6	1 [reference]
July 2001 – June 2007	84	18	3737.4	4.8	1.5 (0.8-2.8)
Mechanistic classes					
Hormones	56	3	4885.2	0.6	1 [reference]
Antibodies	44	19	2401.7	7.9	12.1 (3.6-40.9)
Cytokines	4	3	255.3	11.8	17.3 (3.5-86.1)
Enzymes	43	6	3247.0	1.8	2.9 (0.7-11.4)
Growth factors	6	2	344.7	5.8	8.2 (1.4-49.1)
Interferons	11	4	884.0	4.5	7.3 (1.6-32.8)
Receptors	3	2	69.0	29.0	34.2 (5.6-211.1)
Others/ various	7	2	579.4	3.5	4.9 (0.8-29.6)
Monoclonal antibodies					
Humanised	15	11	482.4	22.8	1 [reference]
Chimeric	4	4	62.5	64.0	2.9 (0.9-9.9)
Murine	8	2	827.2	2.4	0.2 (0.03-0.7)

a) Not all biologicals could be classified according to position in class and these biologicals were therefore excluded from the analysis.

Table 6: Nature of the safety-related regulatory actions classified at System Organ Class (SOC) level specified by safety-related regulatory action[a].

System Organ Class Level	No. (%) of safety-related regulatory actions
Dear Healthcare Professional letter US	
General disorders and administration site conditions	11 (16.2)
Infections and infestations	7 (10.3)
Blood and lymphatic system disorders	6 (8.8)
Immune system disorders	6 (8.8)
Others	38 (55.9)
Direct Healthcare Professional Communication EU	
General disorders and administration site conditions	6 (21.4)
Infections and infestations	6 (21.4)
Blood and lymphatic system disorders	3 (10.7)
Others	13 (46.4)
Black box warning US	
General disorders and administration site conditions	5 (14.7)
Infections and infestations	5 (14.7)
Immune system disorders	5 (14.7)
Neoplasms benign, malignant and unspecified	5 (14.7)
Others	14 (41.2)

a) Multiple primary safety warnings issued at the same time were included as multiple events.
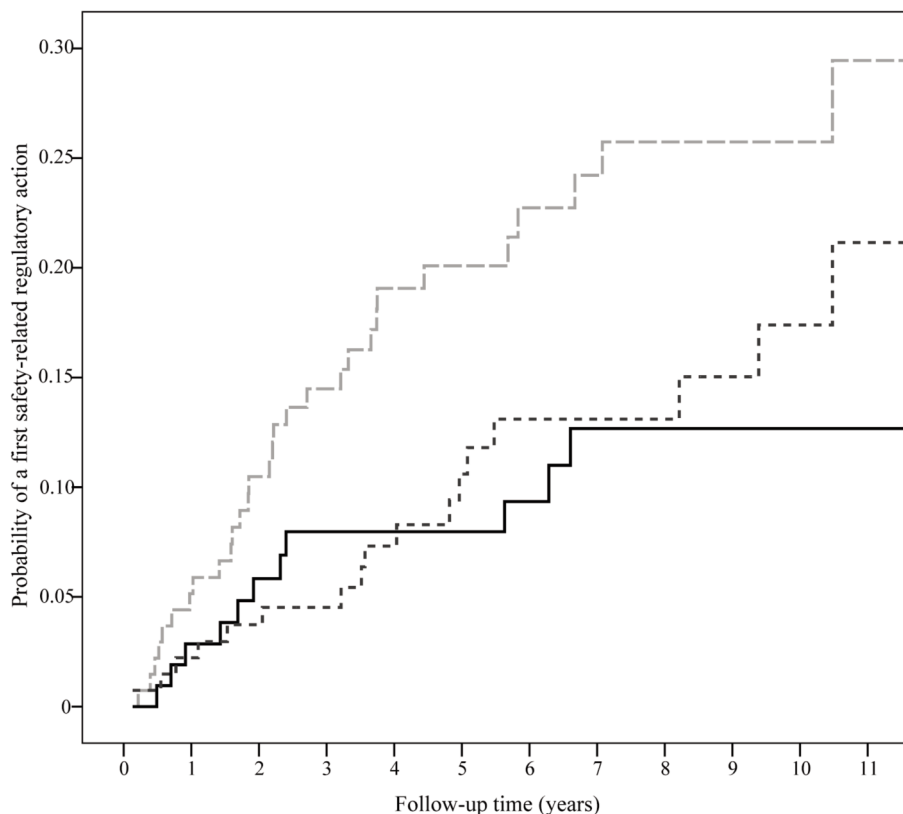
A significantly higher risk for a first safety-related regulatory action compared with hormones was found for antibodies (HR 12.1; 95% CI 3.6-40.9), cytokines (HR 17.3; 95% CI 3.5-86.1), growth factors (HR 8.2; 95% CI 1.4-49.1), interferons (HR 7.3; 95% CI 1.6-32.8), and receptors (HR 34.2; 95% CI 5.6-211.1). Compared with humanised monoclonal antibodies, chimeric monoclonal antibodies had a non-significantly higher risk (HR 2.9; 95% CI 0.9-9.9) and murine monoclonal antibodies had a lower risk (HR 0.2; 95% CI 0.03-0.7) for a first safety-related regulatory action (Table 5).

The safety-related regulatory actions issued for biologicals mostly concerned the system organ classes of general disorders and administration site conditions (26.8% of 82), infections and infestations (22%), immune system disorders (15.9%), and neoplasms benign, malignant, and unspecified (12.2%) (Tables 2, 3, and 4). A quantitative description of the frequency of each type of safety-related regulatory action is shown in Table 6.

The 46 DHPLs, 17 DHPCs, and 19 black box warnings related to safety were issued for 30 (22.1%), 11 (10.5%), and 17 (12.6%) different biologicals, respectively. After 10 years, the probability of a first DHPL was 26% (95% CI 17%-34%); DHPC, 13% (95% CI 5%-20%); and black box warning, 17% (95% CI 8%-26%) (Figure 1). Communications to health care professionals were more frequently issued in the United States compared with the European Union (HR 3.0; 95% CI 1.2-7.6).

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Figure 1: Safety-related regulatory actions for biologicals

Number of biologicals at risk												
Year	0	1	2	3	4	5	6	7	8	9	10	11
United States												
Dear healthcare professional letter	136	129	115	99	86	70	57	50	42	33	26	16
Black box warning	135	133	124	109	97	76	64	56	50	41	31	19
European Union												
Direct healtcare professional communication	105	102	93	82	78	69	56	47	34	24	13	8

The sources of the safety-related regulatory actions described in DHPLs were post-approval reports (n = 18; 39.1%), clinical trial data (n = 16; 34.8%), a combination of post-approval reports and clinical trial data (n = 9; 19.6%), others (n = 2; 4.3%), and unknown (n = 1; 2.2%). The sources of the safety-related regulatory actions described in DHPCs were post-approval reports (n = 9; 52.9%), clinical trial data (n = 5; 29.4%), combination of post-approval reports and clinical trial data (n = 1; 5.9%), others (n = 1; 5.9%), and unknown (n = 1; 5.9%).

Sixty-one safety-related regulatory actions were issued for the subgroup of 67 biologicals approved in both the United States and European Union within the study period. Five safety-related regulatory actions issued in the United States were excluded because the biological was not approved in the European Union at the time of the regulatory action. Nine safety-related regulatory actions issued in both regions involved the same nature (35 were issued only in the United States and 8 were issued only in the European Union). Of the 9 safety-related regulatory actions issued in both regions, 6 were issued in both regions within 2 months (1 was issued first in the United States and 2 were issued first in the European Union).

Comment

The experience with drugs in actual clinical practice complements that of clinical trials and helps to expand the knowledge of the safety profile of drugs.[7,23,24] Biologicals are a relatively new class of drugs and the expected safety-related regulatory actions issued in the system organ class of infections and infestations relating to the immunomodulatory effect of many of the biologicals was confirmed by the present study. The safety-related regulatory actions issued in the system organ class of general disorders and administration site conditions can be partly explained by the infusion reactions occurring after the parenteral route of administration, which is the mode of administration for most biologicals. A more in-depth evaluation of the mode of action of biologicals might have predicted some safety problems during the developmental phase. Tumour necrosis factor, for example, is released by activated macrophages, T lymphocytes, and other immune cells and plays an important role in the human immune response to infections.[27,28] As shown by the present study, the risk of infections with the tumour necrosis factor antibody infliximab was identified and communicated after approval of the drug. The association between non-steroidal anti-inflammatory drugs and the occurrence of gastrointestinal tract adverse events, however, illustrates that the need for an in-depth evaluation of the mode of action counts also for small molecules.[29]

There are limited data available in the literature on the timing and frequency of safety-related regulatory actions issued post-approval. The present study showed that the 174 biologicals had a probability of 14% to require their first safety-related regulatory action within 3 years after receiving marketing authorisation, and the probability increased to 29% within 10 years after approval. In this context, it is important to notice that not all drugs are marketed immediately after approval and some drugs will never be marketed. Because all biologicals that obtained market authorisation were included in the present study, this may have led to an underestimation of the probability of a safety-related regulatory action, which is a limitation of this study. Unfortunately, marketing status could

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not be retrieved from the US Food and Drug Administration and the EMEA web sites. Lasser et al[23] found that new chemical entities approved until 1999 had a probability of a black box warning of 10% after 10 years of marketing compared with 17% found in our study. Biologicals, therefore, seem to be more susceptible to a black box warning issued post-approval compared with new chemical entities in general. However, existing differences between both studies, with the inclusion of multiple black box warnings issued for the same drug and a delay in the occurrence of a black box warning in the *Physicians' Desk Reference* by Lasser et al,[23,30] and an increasing awareness of patient safety and accessibility of safety data over time,[31] preclude a direct comparison of probabilities.

A limitation that should be addressed is the relatively small sample size and the small number of safety-related regulatory actions resulting in broad 95% CIs, which makes interpretation of non-statistically significant findings challenging. However, it was decided to only include biologicals from 1995 onward because centralised decision making by the EMEA for all biologicals started in 1995.

As shown in this study, the decision for a safety-related regulatory action can be based on (large) clinical trials, case reports, and/or epidemiological studies and thus also may be based on clinical observations without any formal epidemiological or experimental confirmation. In addition, the number of patients exposed to the different drugs may vary greatly, which may affect both detection of the safety problem as well as its significance for clinical practice. This dilemma in decision making – availability of incomplete data and variability in patient exposure – is acknowledged. However, dissemination of a safety-related regulatory action is usually the result of a balanced assessment by the regulatory authorities taking into account the seriousness of the safety problem and the need to inform health care professionals. Large epidemiological studies and/or clinical trials are often needed to confirm the association between the safety problem resulting in a regulatory action and the drug. The effect of epidemiological studies for the identification of safety problems could not be taken into account in this study because it was not possible to differentiate between safety problems identified by spontaneous reports and results from epidemiological studies based on the communications to health care professionals. Some of the biologicals (for example, monoclonal antibodies) differ essentially from naturally occurring substances and might therefore be especially susceptible to adverse drug reactions.[26] Although the 95% CIs were broad, our study confirmed that these and other biologicals, including cytokines, growth factors, interferons, and receptors were specifically prone to safety-related regulatory actions. Within the group of monoclonal antibodies, the murine antibodies had a lower risk for a first safety-related regulatory action compared with the humanised monoclonal antibodies. However, this finding should be interpreted with caution due to the small number of monoclonal antibodies and safety-related regulatory actions.

The first biologicals approved in a chemical, pharmacological, and therapeutic subgroup were at a higher risk for their first safety-related regulatory action. This finding suggests that pharmacovigilance should especially be stringent for the first biologicals to be approved in a chemical, pharmacological, and therapeutic subgroup. Our study also showed that biologicals approved during the last 6 years of the study period (July 2001 to June 2007) had a non-significant higher risk for their first safety-related regulatory action com-

pared with biologicals approved during the first 6.5 years of the study period (January 1995 to June 2001). This higher risk is mainly due to the high number of DHPLs issued in 2005. Most of the biologicals approved in a new chemical, pharmacological, and therapeutic subgroup were approved during the first 6.5 years of the study period, which does not explain the higher risk for the first safety-related regulatory action for biologicals approved during the last 6 years of the study period.

Differences exist in the nature of the safety-related regulatory actions for biologicals compared with small molecules. As known from previous studies, most of the safety-related problems identified post-approval for the small molecules are identified in the system organ classes of hepatobiliary disorders, blood and lymphatic system disorders, cardiac disorders, and nervous system disorders.[23,24,32] Knowledge on the nature of the safety events and the difference between the small molecules and the relatively new biologicals seems relevant. Lack of awareness of the nature of the safety issues related to the biologicals might hamper the link with the biological and its adverse event when a patient presents with a clinical problem.

More letters were disseminated in the United States compared with the European Union, which is in line with a previous observation that the approach to safety information appeared to be more conservative in the EU summary of product characteristics compared with the US package insert.[33] In both regions, the dissemination of a letter can be initiated by the marketing authorisation holder and by the authorities when important safety information has been identified and when important changes have been made to the product labeling.[34,35] In addition, in the United States a letter can be issued to emphasise corrections to a prescription drug advertisement or to labelling as well.

Only 67 biologicals were approved in both regions within the study period. It should be noticed that some biologicals may have been approved in the other region prior to the study period. Of the 56 safety-related regulatory actions issued for these 67 biologicals, only 9 safety-related regulatory actions involved the same type of safety warning. This seems relevant because the other 35 and 8 safety-related regulatory actions issued in the United States and the European Union, respectively, were not issued in the other region. Because only the most recently approved summary of product characteristics were available from the EMEA web site, it was not possible to investigate if adverse drug reactions communicated by a DHPL in the United States were already included in the EU summary of product characteristics or were added to the EU summary of product characteristics without communication by a DHPC. Six of the 9 warnings issued in both regions were issued within a period of 2 months.

In summary, this study has shown that almost 50% of the safety-related regulatory actions for biologicals were issued in the system organ classes of general disorders and administration site conditions, and infections and infestations. Warnings issued in the system organ class of infections and infestations were often related to the immunomodulatory effect of many biologicals. Although the limitations of preclinical trials for biologicals are acknowledged, results from pharmacology studies, preclinical studies, and clinical studies might result in the prediction of potential risks related to the drug for which close monitoring is needed in the post-approval setting. Health care professionals should be

aware of the specific risks related to the relatively new class of biologicals to be able to provide a link between the use of the biological and the patient presenting with a clinical problem. In addition, the classes of antibodies (monoclonal), cytokines, growth factors, interferons, and receptors and the first biologicals approved in a chemical, pharmacological, and therapeutic subgroup are specifically prone to a first safety-related regulatory action; close monitoring of these biologicals is therefore recommended.

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Chapter 2.3

Safety-related regulatory actions for orphan drugs in the United States and the European Union: a cohort study

Harald E. Heemstra
Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Remco L.A. de Vrueh
Hubert G.M. Leufkens

Chapter 2.3

Abstract

Background: Drugs for rare diseases, so-called orphan drugs, are often intended for serious or chronically debilitating diseases. Safety information is more limited at the time of approval for orphan drugs as a result of various factors, such as the limited number of patients in clinical trials, quality of the clinical trials and special approval procedures. Several studies have been conducted on safety-related regulatory actions for drugs, but none of these specifically focused on orphan drugs.

Objective: To determine the frequency and nature of safety-related regulatory actions for orphan drugs in the US and EU.

Methods. This cohort study examined publicly available data from the web sites of US and EU regulatory authorities on orphan drugs approved in the US and/or the EU between January 2000 and December 2007. The main outcome measures were the nature, frequency and timing of safety-related regulatory actions, defined as (1) safety withdrawals; (2) black-box warnings; and (3) written communications to healthcare professionals issued by the US FDA or the European Medicines Agency between January 2000 and June 2008.

Results: Ninety-five orphan drugs were approved during the study period (75 in the US, 44 in the EU, and 24 in both regions). Ten products (10.5%) received a safety-related regulatory action. No safety withdrawals, four black-box warnings and 12 written communications were identified. The probability of a first safety-related regulatory action for orphan drugs was 20.3% after 8 years of follow-up. Orphan drugs approved by accelerated approval (relative risk [RR] 3.3; 95% CI 1.1-10.4), oncological products (RR 7.8; 95% CI 1.0-63.8) and products for gastrointestinal and metabolism indications (RR 10.4; 95% CI 1.2-87.3) may have a higher risk for a safety-related regulatory action.

Conclusions: The probability of a first safety-related regulatory action for an orphan drug was slightly lower than that reported in the literature for biologicals in one study and new molecular entities in another study. However, detection of safety issues may be complicated by the limited experience with orphan drugs in practical use due to the low prevalences of the diseases they are used for. Doctors and pharmacists should therefore be vigilant with regard to the occurrence of a safety-related issue for orphan drugs.

Background

Orphan drugs are drugs indicated for the treatment, prevention or diagnosis of rare diseases. The number of approved orphan drugs is growing steadily since the enactment of dedicated orphan drug regulations in both the US[1] and the EU.[2] These drugs are often intended for serious or chronically debilitating diseases, for which no suitable treatment has previously been approved. In the EU, these are criteria that are required to become designated as an orphan drug,[2] whereas in the US about 85% of orphan drugs are being used for serious and/or life-threatening diseases.[3] In contrast to other drugs, these drugs are intended for use in smaller populations and, moreover, the severity of the diseases means that there is usually a high medical need for treatments for these indications.

Because of the small numbers of patients, clinical trials are often conducted with few subjects.[4-7] Therefore, the clinical development of these drugs may not have been performed as thoroughly as that for other drugs. Clinical experience of an orphan drug at the time of marketing may thus be fairly limited, with the result that knowledge on the safety profile of approved orphan drugs may be less than that for other drugs.[8-10] Consequently, unexpected safety issues may emerge more frequently during use in daily practice for orphan drugs compared with other drugs. Table 1 lists certain characteristics of orphan drugs that may affect the likelihood of a safety-related event. However, the high medical need for most of these orphan drugs apparently justifies approval of the product based on the available data. As with other drugs, the safety of an orphan medicinal product is carefully monitored post-approval and, if necessary, regulatory authorities will take actions to protect public health.

Table 1: Characteristics of orphan drugs that may affect the likelihood of a safety-related event.

- Many orphan drugs are biologicals or new chemical entities based on innovative new molecules or delivery mechanisms[3,11,12] that may have a higher chance of unexpected safety events.[9]
- Many orphan drugs are intended for the treatment, diagnosis or prevention of chronic or serious diseases in patients that may be more prone towards adverse events.[3]
- Many orphan drugs are approved based on very small-scale clinical trials, and clinical experience with orphan drugs is often limited at the time of marketing approval. Consequently, knowledge of the safety of the product is limited.[4,5]
- Only 57% of approved orphan drugs have been tested in a randomized clinical trial before approval.[5] In addition, Joppi et al.[4,5] describe several other deficiencies on the clinical development of orphan drugs, including lack of active controls, use of incorrect surrogate parameters and duration of trials that are too short, all of which contribute to the limited availability of clinical experience with an orphan drug.
- Large numbers of orphan drugs are approved as an accelerated approval (US), under exceptional circumstances (EU) or as a conditional approval (EU), possibly limiting the availability of pre-approval safety information.[5]

Studies investigating the frequency and nature of post-approval safety-related regulatory actions have been conducted by Giezen et al.[13] in 2008 and Lasser et al.[14] in 2002. Another study evaluated post-authorisation safety studies as proposed at the time of regulatory approval.[15] None of these studies focused on orphan drugs. However, within the study by Giezen et al.,[13] 17 of 30 (57%) biologicals that obtained a 'Dear Healthcare Professional' letter (DHPL) from the US FDA, and 11 of 17 (65%) biologicals with a black-

box warning were orphan drugs, whereas none of the biological orphan drugs received a direct healthcare professional communication (DHPC) from the European Medicines Agency (EMEA).[13] In the study by Lasser et al.,[14] 6 of the 45 (13%) 'black-box' warnings for new molecular entities were issued for FDA-approved orphan drugs; [14] however, comparable figures on the entire group of orphan drugs are not known.

In this study, we therefore aim to determine the frequency and nature of safety-related regulatory actions for all orphan drugs approved in the US and/or EU from the initiation of the EU Regulation on Orphan Medicinal Products in January 2000.[2] Moreover, we determine whether the occurrence of safety-related regulatory actions is related to the type of molecule (biological or small molecule), type of approval and indication class.

Methods

We have included all medicinal products with an orphan designation in the US, the EU, or both regions, that have been approved for their first indication between the enforcement of the EU 'Regulation on Orphan Medicinal Products' in January 2000 to January 2007 and for which their first approved orphan indication was also within this time period. An orphan indication was defined in this study as an indication forthcoming from an official orphan designation by the FDA or the EMEA. For most orphan drugs, the first registration is also the registration for an orphan indication, but some drugs were approved for another indication before approval for an orphan indication.

For the period January 2000 to June 2008, important safety-related regulatory actions that required urgent communication were identified, such that, for all products, at least 6 months follow-up time could be observed. Safety-related regulatory actions were classified as follows: 1) safety-related market withdrawals (US and EU); 2) Post-approval black-box warnings (US); 3) Written communications to healthcare professionals (DHPLs [US only] or DHPCs [EU only]). DHPLs were identified from MedWatch [www.fda.gov /medwatch], and DHPCs were identified from the web site of the Medicines Evaluation Board in the Netherlands [www.cbg-meb.nl] and European Public Assessment Reports of the EMEA [www.emea.europa.eu]. The date of the letter was used as the date of the safety-related regulatory action. Letters not including safety warnings and follow-up letters of previously issued letters containing no new safety information were excluded.

Post-approval black-box warnings were identified from the labels available from the web sites of the FDA Center for Drug Evaluation and Research (CDER) [www.accessdata .fda.gov/scripts/cder/drugsatfda/index.cfm], MedWatch and the marketing authorisation holder. The latest approved label of every orphan drug available from the CDER was searched for a black-box warning. Where a black-box warning was identified from the latest approved label, previously approved labels were checked to identify the date the black-box warning was added, which was cross-checked with the information from Med-Watch. The date of the black-box warning stated on the web sites of the CDER or Med-Watch was included in the analysis. Where the exact date of the black-box warning could not be identified, the latest possible date the black-box warning was issued was included in the analysis.

The nature of the safety-related regulatory action was coded at the System Organ Class (SOC) level according to the Medical Dictionary for Regulatory Activities (MedDRA) version 11.1. Moreover, for each of the selected products, information was collected on the following variables: region of approval, type of molecule, approval circumstances and indication of the product. For the region of approval, products could be approved in the US, the EU or both regions. The type of molecule could be biological (extracted or produced from living organisms[11]) or a small molecule**.** Two groups of approvals were defined: (i) accelerated approvals, defined as accelerated approvals (US only), conditional approvals or approvals under exceptional circumstances (both EU only); and (ii) other approvals. Finally, data on time between the first approval (orphan or regular) and the regulatory action was determined. For every unique approved product, only the data on the first approval were included in the study. Unique products were defined as products with the same active compound, indication and sponsor.

Kaplan-Meier survival curves were plotted to estimate the probability of the occurrence of a first safety-related regulatory action for the total group of orphan drugs, and by region (US and EU) and type of warning (written communications and black-box warnings). The proportional hazard assumptions proved not to be valid for some of the variables of interest. Therefore, cumulative incidence rates at the end of the study after 8.5 years, relative risks (RR) and corresponding 95% confidence intervals (CIs) were calculated for the risk of a first safety-related regulatory action for the orphan drugs with each of the above-mentioned variables.

Results

Between January 2000 and December 2007, 75 orphan drugs were approved in the US and 44 in the EU. Of these products, 24 were approved as orphan drugs in both regions, resulting in a total group of 95 unique products. Median follow-up time was 3.7 years (range 0.6–8.3 years). For ten (10.4%) of the products included in the study, one or more safety-related regulatory actions were identified. No safety-related withdrawals were observed during the study period. Of the 75 products that were approved in the US, seven products obtained a total number of ten DHPLs. In addition, two of these products and two other products received a black-box warning (Table 2). Of the products that obtained a regulatory action, sodium phenylacetate/sodium benzoate (Ammonul®) obtained a DHPL with a warning for particulate matter in the infusion product, while bosentan (Tracleer®) obtained a DHPL reminding physicians of the importance of liver function tests. The remaining seven products received boxed or written warnings regarding newly detected safety risks, such as infusion reactions and immunological reactions. Of the 44 products that were approved in the EU, two products received a total of three DHPCs by the EMEA, of which two were for imatinib (Gleevec®/Glivec®), which also received a DHPL from the FDA (Table 2).

Table 2: Nature and timing of orphan drugs with a post-approval, safety-related regulatory action in the US and EU.

Active substance	Registered trade name	Approval date for first orphan indication	Warning	System organ class	Time to safety related regulatory action (y)	Region of marketing approval	Safety issue included in SPC (EU) or label text (US)
Black-box warnings							
Cetuximab	Erbitux®	12 February 2004	Cardiopulmonary arrest	Cardiac disorders	2.0	US and EU[a]	In SPC since 27 February 2007
Gemtuzumab ozogamicin	Mylotarg®	17 May 2000	Hypersensitivity reactions, including anaphylaxis, infusion reactions, pulmonary events/ hepatotoxicity	Immune system disorders/ hepatobiliary disorders	0.8	US	
Ibritumomab tiuxetan	Zevalin®	19 February 2002	Severe cutaneous and mucocutaneous reactions	Skin and subcutaneous tissue disorders	3.6	US and EU[a]	In SPC since 15 November 2005
Laronidase	Aldurazyme®	30 April 2003	Life-threatening anaphylactic reactions	Immune system disorders	5.0	US and EU	Included in original SPC, 10 June 2003
Dear Healthcare Professional Letters							
Sodium phenylacetate/ Sodium benzoate	Ammonul®	17 February 2005	Detection of particulate matter in injection product	Injury, poisoning and procedural complications	3.6	US	
Bosentan	Tracleer®	20 November 2001	Reminder of the importance of monthly liver function testing due to cases of hepatotoxicity	Investigations	4.3	US and EU	Included in original SPC, 15 May 2002

Cetuximab	Erbitux®	1 March 2006	Hypomagnesia/ infusion reactions	Metabolism and nutrition disorders/ general disorders and administration site conditions	1.6	US and EU[a]	Included in SPC since 13 September 2005, updated 27 February 2006
Deferasirox	Exjade®	2 November 2005	Risk for renal failure	Renal and urinary disorders	1.5	US and EU	Included in original SPC, 28 August 2006
			Risk for hepatic failure	Hepatobiliary disorders	2.1		Included in SPC since 25 July 2008
Ibritumomab tiuxetan	Zevalin®	19 February 2002	Severe cutaneous or mucocutaneous reactions	Skin and subcutaneous tissue disorders	3.7	US and EU[a]	In SPC since 15 November 2005
Imatinib	Gleevec®	10 May 2001	Severe congestive heart failure and left ventricular dysfunction	Cardiac disorders	5.4	US and EU	Direct Health Professional Communication on 11 December 2006
Zoledronic acid	Zometa®	20 August 2001	Dosage adjustments in patients with multiple myeloma and metastatic bone lesions from solid tumours	Surgical and medical procedures	3.1	US and EU[a]	Included in original SPC, 20 March 2001
			Osteonecrosis of the jaw	Musculoskeletal and connective tissue disorder	3.7		Included in original SPC, updated on 6 January 2006
			Atrial fibrillation	Cardiac disorders	6.1		Included in SPC since 19 October 2007

Table 2: Continued.

Active substance	Registered trade name	Approval date for first orphan indication	Warning	System organ class		Region of market-ing approval	Safety issue included in SPC (EU) or label text (US)
Direct Healthcare Professional Communications							
Imatinib	Glivec®	7 November 2001	Preclinical carcinogenicity findings	Neoplasms be-nign, malignant and unspecified	3.4	US and EU	Included in the label since 16 December 2004
			Heart failure and leftventricular dysfunction	Cardiac disorders	5.1		Dear Healthcare Professional Letter on 19 October 2006
Miglustat	Zavesca®	20 November 2002	Preclinical carcinogenicity findings in rats and mice	Neoplasms be-nign, malignant and unspecified	4.4	US and EU	Included in the label since 29 February 2008

a) Indicates the drug is approved in the EU but has not obtained orphan status.
SPC = summary of product characteristics.

In addition, of the 13 safety-related regulatory actions unique for US-approved products, five of the underlying safety-issues were already included in the original summary of product characteristics (SPC), while six resulted in a change in the SPC around the time of the regulatory action and two were for products not approved in the EU. Both safety-related regulatory actions unique for EU-approved products resulted in a change in the US label text (Table 2).

Safety-related regulatory actions for the orphan biologicals included two actions classified at the SOC level as immune system disorders, and two as skin and subcutaneous tissue disorders, although the latter involved one product. Actions for the small molecule orphan drugs included three actions classified at the SOC level as cardiac disorders and two as neoplasms, benign, malignant and unspecified (Table 2).

The overall probability for obtaining a first safety-related regulatory action for orphan drugs was 3.5% after 3 years of follow-up and 20.3% after 8 years of follow-up. Figure 1 shows the probabilities to obtain a first safety-related action (written communication or black-box warning) for orphan drugs approved in the US or EU. For the orphan drugs approved in the US, the probability, after 6 years, to obtain a first DHPL was 18.6% and for a black-box warning, the probability was 10.0%. For orphan drugs approved in the EU, the probability of a DHPC after 6 years was 13.6%.

Table 3 provides an overview of the characteristics of the orphan drugs included in the study. Biological orphan drugs do not have a statistically significantly higher RR to obtain a safety-related regulatory action compared with small molecules (RR 1.7; 95% CI 0.5-5.5). Of the 95 orphan drugs in the study, 22 were under accelerated conditions, either as an accelerated approval (n = 11) by the FDA; or as a conditional approval (n = 2) or an approval under exceptional circumstances (n = 10) by the EMEA. The RR for these products approved in an accelerated course was 3.3 (95% CI 1.1-10.4) compared with otherwise approved products. Finally, of all ten products with at least one safety-related regulatory action issued by the FDA or the EMEA, five were oncological orphan drugs (Anatomical Therapeutic Chemical [ATC] class L01/L02) and four were indicated for gastrointestinal or metabolic diseases (ATC class A). The RR to obtain a first safety-related regulatory action for oncological products was 7.8 (95% CI 1.0-63.8), and the RR for gastrointestinal and metabolic diseases was 10.4 (95% CI 1.3-87.3) compared with products indicated for other disease classes. In a separate analysis, we compared the RR to obtain a DHPC for the European orphan drugs (n = 44) with the risk of obtaining a DHPL for the US orphan drugs (n = 75). In the current study, it was found that EMEA-approved orphan drugs may have a lower risk (RR 0.5) for a written safety communication than FDA-approved orphan drugs, although this was not statistically significant (95% CI 0.1-2.2).

Chapter 2.3

Figure 1: Kaplan-Meier analysis of the probability to obtain a first safety-related regulatory action for orphan drugs.
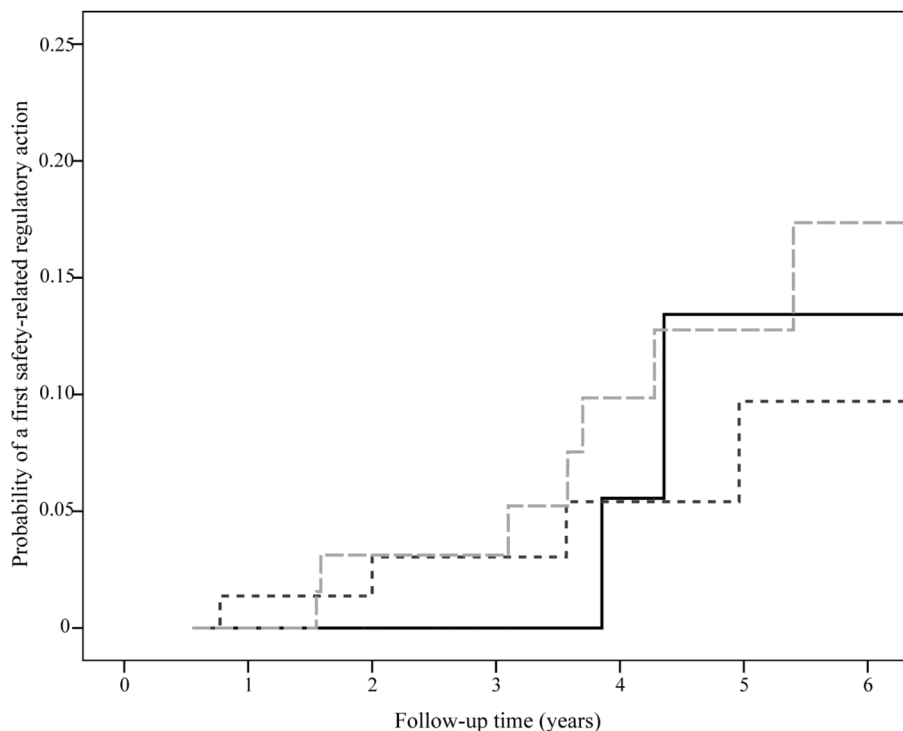
US							
– – –	Black box warning						
― ―	Dear healthcare professional letter						
EU							
———	Direct healthcare professional communication						

Number of orphan drugs at risk							
Year	0	1	2	3	4	5	6
US							
Black box warning	75	70	59	45	33	22	13
Dear healthcare professional letter	75	62	58	46	33	21	11
EU							
Direct healthcare professional communication	44	38	27	20	14	8	4

Table 3: Relative risks and corresponding 95% CIs for the risk of a first safety-related regulatory action for orphan drugs.

Characteristics of selected orphan drugs	Total number of orphan drugs (n = 95)	No. of orphan drugs with a safety-related regulatory action	Median follow-up time [y (range)]	Event rate	Relative risk (95% CI)
Type of molecule					
Small molecule	68	6	3.7 (0.6–8.2)	0.09	1 [reference]
Biological	27	4	3.7 (0.8–8.3)	0.15	1.68 (0.51-5.49)
Approval circumstances					
Normal approval	73	5	3.8 (0.6–8.3)	0.07	1 [reference]
Accelerated approval (accelerated [US only], exceptional or conditional [EU only])	22	5	3.5 (0.8–7.2)	0.23	3.32 (1.06-10.42)
Indication group of the orphan drug					
Other indications	47	1	3.9 (0.8–8.3)	0.02	1 [reference]
Gastrointestinal and metabolism (ATC class A)	18	4	3.9 (0.6–8.0)	0.22	10.44 (1.25-87.27)
Oncology (ATC class L01/L02)	30	5	3.3 (0.7–7.8)	0.17	7.83 (0.96-63.82)
Region of approval[a]					
US	75	7	2.6 (0.6–6.9)	0.09	1 [reference]
EU	44	2	3.7 (0.5–8.3)	0.05	0.49 (0.11-2.24)

a) Limited to written safety communications.
ATC = Anatomical Therapeutic Chemical.

Discussion

In the current study, we found the probability of obtaining a first safety-related event to be 3.5% after 3 years and 20.3% after 8 years for all orphan drugs. We found no statistically significant association between the region of approval of an orphan drug or the type of molecule of the product and the risk for a safety-related regulatory action. However, we did find an association of a higher risk for orphan drugs approved under accelerated circumstances and with orphan drugs intended for gastrointestinal and metabolic indications. In addition, orphan drugs intended for oncological indications may also have an increased risk of a safety-related regulatory action.

Our results indicate a slightly lower frequency and probability of safety-related regulatory actions for orphan drugs than was found for biologicals by Giezen et al.[13] and than that found for new molecular entities by Lasser et al.[14] Giezen et al.[13] found the probability of a first safety-related regulatory action, including written communications to healthcare professionals in the US and the EU, and black-box warnings in the US, was up to 29%, 10 years after the approval, and 27%, 8 years after the approval, for biologicals in general, including orphan biologicals (data on file)[13] Distributed by the type of safety-action, probabilities after 6 years were 23% for a DHPL, 13% for a black-box warning and 10% for a DHPC (data on file).[13] Moreover, Lasser et al.[14] found the probability of a black-box warning or withdrawal due to a safety reason was up to 9% for new molecular entities, 6 years after approval.[14]

Given the severe nature of many of the diseases for which these drugs are indicated, the number of safety-related regulatory actions is relatively low. One may therefore be tempted to conclude that orphan drugs are relatively safe drugs. However, the results presented should be interpreted with caution since practical use is different for orphan drugs compared with other drugs; consequently, knowledge on adverse effects may still be incomplete, even in the years after approval. For orphan drugs, real life utilisation of the product still only involves relatively modest numbers of patients. This is illustrated by the numbers of study subjects in pivotal trials, as reported by Joppi et al.[4] and their estimated treatment populations. For example, two orphan drugs that are available for the treatment of Fabry's disease (agalsidase alfa [Replagal®, EU only] and agalsidase beta [Fabrazyme®]) have been studied in pivotal trials with 41 and 56 patients, respectively.[4] The prevalence of this disease is 1 in 40 000, resulting in an estimated maximum treatment population of 7500 patients in the US and 12 500 in the EU. Another example is the orphan drugs approved for the treatment of pulmonary arterial hypertension, a disease with a prevalence of 6–15 per million.[16] For this disease, five orphan medicinal products were approved in the EU (corresponding number of patients in pivotal trials): ambrisentan (Volibris®, 261 patients); bosentan (Tracleer®, 32 patients); iloprost (Ventavis®, 203 patients); sildenafil (Revatio®, 278 patients); and sitaxentan (Thelin®, 516 patients).[4,17] It should be noted that the number of patients actually treated with an orphan drug is usually much lower than the calculated prevalence of the indication.[18] As a consequence of this, the chances of finding serious adverse drug effects during clinical use are not very high and, consequently, safety-related regulatory actions may be taken later than would be expected.[9,10] Thus, the fact that we find lower frequencies of safety-related regulatory actions does not necessarily mean that the risks are less for orphan drugs.

This is best illustrated by the two ATC classes of orphan drugs that we identified with a higher risk for a safety-related regulatory action compared with orphan drugs in other ATC classes (oncological indications; gastrointestinal and metabolic indications). These two classes are being represented by drugs with a relatively high utilisation. Oncological orphan drugs are a special class in this respect. Although indicated for rare indications, these drugs are generally focused on a wide range of rare oncological indications, in trials before approval, but also after approval by way of indication extensions for other rare oncological indications. In addition, many of these products are frequently used for off-label indications, which further increases clinical experience.[12] An important example in this group, imatinib (Gleevec® [US]/Glivec® [EU]) has been on the market in the US since May 2001 and in the EU since November 2001. From that time, the initial indication has been extended to encompass a wide range of oncological indications and thus it is being used in the treatment of large numbers of patients.[19] The amount of clinical experience that has been built with this drug is therefore relatively large.[20] It is the only orphan drug on the market in both the EU and the US that obtained a safety warning in both regions, a DHPL in the EU and a DHPC in the US. For orphan drugs that are used by large patient populations, the chances of detecting any safety issues are higher. This illustrates the relationship between clinical experience with a drug and the identification of risk and thus the obtaining of a safety-related regulatory action.

Moreover, a large number of orphan drugs have been approved as an accelerated approval by the FDA, or under exceptional circumstances or as a conditional approval by the EMEA. These are all dedicated approval programmes aimed at making available promising products for life threatening diseases, based on preliminary evidence prior to formal demonstration of patient benefit.[21,22] Therefore, these approvals usually involve a high number of postmarketing obligations in which the sponsor must demonstrate safe and efficacious use of the product for the intended indication in a larger than normal number of patients. The higher risk of these products for a safety-related regulatory action may therefore be a consequence of both the even more limited knowledge on the safety profile of these products and the close monitoring of use in daily practice, as part of the post-marketing obligations for these products.

To ensure adequate pharmacovigilance involving orphan drugs, several manufacturers now have dedicated risk-management strategies in place, the aim of which is to ensure a more sensitive detection of adverse events,[23,24] or they are exploring ways of using pharmacogenomics as part of pharmacovigilance,[25] while other manufacturers base their risk management strategies on spontaneous reporting of adverse events.[24] Further improving dedicated pharmacovigilance for orphan drugs will be the next step that we will hopefully be able to explore further in the upcoming years. Recently presented plans by the EMEA and FDA to improve pharmacovigilance and risk management may play a role in this.[26-28]

The relatively large share of safety-related regulatory actions classified as immune system disorders for orphan biologicals is also observed by Giezen et al.[13] in their study on biologicals. Moreover, most regulatory actions for small-molecule orphan drugs were classified as cardiac disorders, which correlate to the findings for new molecular entities as

found by Lasser et al.[14] Consequently, based on the limited data available, the nature of safety-related regulatory actions for orphan drugs does not seem to be different from other, non-orphan drugs.

Lasser et al.[14] suggest that clinicians should be reluctant to use new drugs when older, safer alternatives are available, and that patients should be informed about the risks of new drugs. This is exactly the challenge that clinicians face when treating a patient with an orphan drug. The same applies to regulators when assessing a potential orphan drug. However, alternatives are usually not available for the treatment of serious and chronically debilitating rare diseases. Consequently, the assessment of benefits and risks for an orphan drug may be more positive given the amount of information available compared with that for regular drugs. The large number of accelerated approvals for orphan drugs also illustrates this. The fact that a treatment is available for a specific rare disease is often already a large step forward for doctors and patients.

A number of limitations of the present study should be addressed. First, the relatively short duration of follow-up for some of the products, the relatively small sample size and, consequently, the small number of safety-related regulatory actions identified, resulting in broad and non-significant 95% CIs, makes interpretation of non-statistically significant findings challenging. However, it was decided only to include orphan drugs from the year 2000 onwards because that was the time the European Regulation on Orphan Medicinal products commenced and, consequently, the presented data are all that was available from that time. Second, definitions of orphan drugs differ slightly between the US and the EU, which may partly explain the larger number of users of orphan drugs in the US and subsequent relatively larger number of safety warnings. Third, the lower numbers of safety-related regulatory actions in the EU may also be caused by the fact that in the EU, a number of the underlying safety issues were already included in the SPC at the time of approval, or were updated in the SPC when the safety issue became known. The latter indicates a different risk perception by the regulatory authorities, for which no urgent action was needed. Finally, these changes in the SPC (EU) or drug labels (US, except black-box warnings) have not been included in this study. The reason for this was that we were interested in the major safety-related regulatory actions issued, which are, in our opinion, covered by withdrawal of the product, a black-box warning in the US, or the dissemination of a DHPL in the US or DHPC in the EU. These additional safety warnings were therefore beyond the scope of the study.

Conclusion

This study has determined the nature and frequency of safety-related regulatory actions for orphan drugs in the US and the EU. Although we found slightly lower frequencies and probabilities of safety-related regulatory actions for orphan drugs than those presented for biologicals in one study or new molecular entities in another study, it is clear from the above that these actions issued on orphan drugs are just the tip of the iceberg. Issuing safety-related regulatory actions is based on the early detection and communication of safety issues. The lower utilisation of these drugs, however, also means that the chances for detection of a safety issue are lower. The slightly lower numbers of safety-related regulatory actions reported for orphan drugs therefore do not indicate a greater safety for or-

phan drugs. In particular, orphan drugs approved in an accelerated procedure and orphan drugs indicated for oncological and gastrointestinal and metabolic indications have an increased risk for a safety-related regulatory action. However, these may be explained by higher utilisation or monitoring and, consequently, higher chances for detection of a safety issue, of these groups of drugs. Doctors and pharmacists should therefore be vigilant with regard to the chance of occurrence of a safety-related issue for orphan drugs.

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Chapter 3

Evaluation and classification of adverse drug reactions in the clinical risk management of biologicals

Chapter 3.1

**Mapping the safety profile of biologicals:
a disproportionality analysis using the WHO
adverse drug reaction database, VigiBase**

Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Ronald H.B. Meyboom
Sabine M.J.M. Straus
Hubert G.M. Leufkens
Toine C.G. Egberts

Chapter 3.1

Abstract

Background: Biologicals have specific characteristics, as compared with the small molecule drugs, and carry specific risks. Safety problems, for example infliximab and the risk for tuberculosis, have been identified via spontaneous reports of suspected adverse drug reactions (ADRs). However, in general there is limited data on the nature of spontaneously reported suspected ADRs for biologicals.

Objective: To map the safety profile of biologicals as compared with all other drugs. In addition, mechanistic classes of biologicals will be compared.

Methods: Data was obtained from the ADR database (VigiBase) maintained by the WHO Collaborating Centre for International Drug Monitoring. A disproportionality analysis was performed in which case reports for biologicals and all other drugs (the reference group), reported between January 1995 and December 2008 were selected. Vaccines were not included in the analysis. Suspected ADRs were classified according to MedDRA® version 12.0 at the System Organ Class (SOC) level. Biologicals were classified into mechanistic classes: antibodies, cytokines, enzymes, growth factors, hormones (reference group), interferons, receptors and others/various. The safety profile of the biologicals versus all other drugs in the database and of the various mechanistic classes of biologicals was compared using the proportional reporting ratio (PRR).

Results: 191,004 case reports containing 546,474 suspected ADRs were reported for 62 different biologicals, and 2,556,209 case reports containing 8,761,522 suspected ADRs were reported for all other drugs (the reference group). It was found that two-thirds of all suspected ADRs reported for biologicals were reported for five active substances: etanercept (20.3%), interferon-β-1a (15.6%), infliximab (11.6%), teriparatide (10.7%) and adalimumab (9.0%).

Comparison of the safety profile of biologicals and the reference group showed that suspected ADRs for biologicals were more frequently reported in the SOCs 'Infections and infestations' (PRR 4.5), 'Surgical and medical procedures' (PRR 2.4) and 'Neoplasms benign, malignant and unspecified' (PRR 2.1), and less frequently reported in the SOCs 'Psychiatric disorders' (PRR 0.4), 'Vascular disorders' (PRR 0.4) and 'Pregnancy, puerperium and perinatal conditions' (PRR 0.4).

Regarding the differences in safety profile between various mechanistic classes of biologicals, compared with hormones (reference group), 'Infections and infestations' were more frequently reported for receptors and antibodies. 'Neoplasms benign, malignant and unspecified' were more frequently reported for antibodies, cytokines, interferons and receptors, and less frequently for enzymes as compared with the reference group.

Conclusions: In VigiBase, five biologicals comprise two-thirds of the suspected ADRs reported for biologicals, which might distort the relation found between a specific biological and a specific adverse event in case of quantitative signal detection. Therefore the choice of reference group to be used in case of quantitative signal detection should be considered very carefully.

This study confirmed that biologicals have a different safety profile compared with all other drugs in the database and, within the group of biologicals, differences exist between

mechanistic classes. Infections are, for example, frequently reported for receptors and antibodies, which often have an immune compromising effect. Such predictable safety issues should be specifically studied by pre-registration clinical trials and/ or targeted pharmacovigilance. In addition, since not all adverse reactions can be predicted or detected during development, spontaneous reporting remains an important tool for the early detection of signals.

Background

Biologicals, also called biopharmaceuticals, are important treatment options for a variety of chronic and sometimes life-threatening diseases.[1] However, compared with the traditional chemically synthesised small molecules, biologicals have specific characteristics that influence their safety profile. For example, biologicals are large, complicated molecules with a very complex production and purification process, a high potential for immunogenicity and limited predictability of preclinical data to clinical outcomes.[1-4] These characteristics may result in more uncertainties about the safety profile of biologicals at the point of approval, which was confirmed by a previous study. In this study it was shown that, at the moment of marketing, safety concerns for biologicals are less frequently classified as 'identified risks' and more frequently as 'missing information' compared with the small molecule drugs.[5]

Because of the limitations of randomised clinical trials, including the often limited sample size and a homogenous population, pharmacovigilance is required to further study the safety of drugs in the postmarketing setting.[6] We demonstrated that approximately one in four of all biologicals approved in the US and/ or the EU required a safety-related regulatory action, defined as a communication to healthcare professionals and/ or a 'blackbox' warning, after marketing of the drug.[7] This study also demonstrated that the safety concerns requiring a regulatory action were different between biologicals and small molecules. Safety concerns for biologicals often concerned infections, malignancies and reactions related to immunological events,[7] whereas safety concerns for small molecules are known to be often related to the classes 'Cardiac disorders', 'Hepatobiliary disorders', 'Blood and lymphatic system disorders' and 'Nervous system disorders'.[8-10]

Pharmacovigilance comprises a variety of activities, including the collection of spontaneously reported suspected adverse drug reactions (ADRs) reported by both patients and healthcare professionals, and proactive pharmacovigilance activities, including post-authorisation safety studies.[11] Spontaneous reporting of suspected ADRs has shown to be an important tool for the detection of new, serious and/or rare potential ADRs,[12] although assessment to establish whether the relationship between a drug and a clinical event is causal is complicated.[13] Causality assessment, however, might be even more complicated for biologicals due to a prolonged effect of the biological resulting in the occurrence of potential ADRs weeks or months after administration of the last dose and since patients treated with biologicals are often treated with multiple other drugs and/or have multiple diseases.[4] Two examples illustrate the impact spontaneous reporting of suspected ADRs can have on clinical decision making. First, an analysis in the US FDA spontaneous reporting system MedWatch suggested an association between the occurrence of

tuberculosis with the use of infliximab, which had not been previously seen.[14] Current clinical guidelines now include the recommendation that patients should be tested for latent tuberculosis before treatment with infliximab is initiated and, if latent tuberculosis is shown, this should be eradicated first.[15,16] Second, three confirmed case reports of progressive multifocal leukoencephalopathy with the use of efalizumab, two of which were detected via spontaneous reporting, made the European regulators conclude that the benefits no longer outweigh the risk, which led to withdrawal of the drug from the market.[17] These two examples illustrate the occurrence of a specific ADR with a specific biological. At the moment there is limited data available on the nature of spontaneously reported suspected ADRs for biologicals from a broader perspective and how this relates to the spontaneously reported suspected ADRs for the traditional small molecule drugs. Therefore, the present study aims to further map the safety profile of biologicals, based on spontaneously reported suspected ADRs, as compared with all other drugs and for specific mechanistic subclasses of biologicals. In addition, general characteristics of the spontaneously reported suspected ADRs for biologicals will be collected in terms of (changes in) the number of suspected ADRs reported over time, and the identification of drugs frequently implicated. This study will therefore add to the knowledge on the safety profile of biologicals as compared with the traditional small molecule drugs.

Methods

Setting

The data has been obtained from the WHO Global Individual Case Safety Report (ICSR) database, VigiBase, which is maintained by the Uppsala Monitoring Centre. VigiBase contains summaries of suspected ADR case reports originally submitted by healthcare professionals and patients to national pharmacovigilance centres in 98 countries all over the world. As of May 2010, this database contained over 5 million case reports of suspected ADRs regarding specific, but anonymous, patients. The reports contain details on administrative, patient, ADR and medication data, and additional information. The information in these reports is not homogenous, at least with regard to origin, completeness of documentation or the likelihood that the suspected drug caused the suspected ADR. Suspected ADRs are transferred from the national pharmacovigilance centres to the Uppsala Monitoring Centre and recorded with the lowest level term either in the WHO Adverse Reaction Terminology (WHO-ART) or the Medical Dictionary for Regulatory Activities (MedDRA®), depending on the terminology used at the national centre. Since there is a bridge between the two terminologies in VigiBase these two methods of coding suspected ADRs are compatible and data can be retrieved using either WHO-ART or MedDRA®.[18] In the present study, ADRs were coded according to MedDRA®.

For this study, all suspected ADRs reported to VigiBase between January 1995 and December 2008 for biologicals approved between January 1995 and December 2008 in the EU and/or US were taken into account. Biologicals were defined according to the European Medicines Agency definition, in which biologicals are defined as products that are produced by or extracted from a biological source.[19] The following biologicals were excluded: vaccines (ATC class J07), allergenic products (allergen patch test and allergenic extracts; ATC classes V01AA and V04CL), biological products for further manufacture,

and biological products for transfusion purposes and maintenance of circulating blood volume. All other suspected ADRs reported to VigiBase between January 1995 and December 2008 were used as the reference group (vaccines were also excluded from the reference group).

Design and definition

A disproportionality analysis was conducted in which all suspected ADRs reported between January 1995 and December 2008 for biologicals and the drugs in the reference group were followed over time. Biologicals were classified according to the mechanistic classes: antibodies (including monoclonal antibodies), cytokines, enzymes, growth factors, hormones, interferons, receptors and others/various.[7]

For both biologicals and the reference group, data was obtained on the number of suspected ADRs in the case reports at the lowest level term, changes in the number of reports over time, mean age and sex distribution of the patients. The safety profile of the biologicals and the reference group was characterised and compared according to the spontaneously reported suspected ADRs. Suspected ADRs were classified according to MedDRA® version 12.0 in the primary System Organ Class (SOC). The safety profile was also characterised and compared for the different mechanistic classes of biologicals. Within this analysis, specific attention was given to the MedDRA® SOCs 'Infections and infestations', 'General disorders and administration site conditions', 'Immune system disorders' and 'Neoplasms benign, malignant and unspecified', since our previous study showed that safety-related regulatory actions for biologicals were specifically triggered for these classes of suspected ADRs.[7] Other MedDRA® SOC classes, which were among the five SOCs in which suspected ADRs were most frequently reported in the present study, were also taken into account.

Since infections, neoplasms and ADRs related to immunological events frequently trigger a safety-related regulatory action,[7] these ADRs were evaluated in more depth. Suspected ADRs reported in the SOC 'Infections and infestations' were further classified into opportunistic infections and other infections. Opportunistic infections were defined as an infection caused by an organism that does not normally cause disease, which occurs in patients with weakened immune systems.[20] Suspected ADRs reported in the SOC 'Neoplasms benign, malignant and unspecified' were further classified as haematological malignancies, solid malignancies and others (mainly including benign neoplasms).

Immunological events can range from transient appearance of antibodies without any clinical significance to severe life-threatening conditions. Potential clinical problems include loss of efficacy of the biological due to neutralising antibodies, hypersensitivity reactions and antibodies that cross-react with an endogenous counterpart.[19,21] However, because of the large variety of suspected ADRs that can be considered immunological events, these events were classified as either *definite* immunological or *possible* immunological. Suspected ADRs that were classified as definite immunological events include, for example, hypersensitivity reactions, antibodies to a specific biological, etc., and suspected ADRs that were classified as possible immunological comprise the less specific terms, e.g. fever and hypotension. Immunological events were classified as definite or

possible immunological based on the MedDRA® preferred term level, so ADRs classified in different SOCs were included in this analysis.

Data analysis

Descriptive statistics were used to summarise the general characteristics of the case reports for biologicals and the reference group. The line that best-fitted the cumulative number of case reports over time was calculated using the square of the correlation coefficient. The safety profile of the biologicals and the reference group (all other drugs) was compared by calculation of the proportional reporting ratio (PRR) and their corresponding 95% CI. The PRR is calculated in a similar way to a relative risk in a cohort study, whereby the proportion of a specific ADR or group of ADRs of interest is calculated for biologicals and divided by the proportion of these ADR(s) for all other drugs in the database (reference group) (Table 1).[22,23] In the calculation of the PRR, each suspected ADR classified at the lowest level term was classified at the SOC level and included in the analysis.

Three sensitivity analyses were planned to analyse the effect of specific characteristics on the estimated PRRs. First, an analysis was done after exclusion of the five biologicals most frequently implicated. Second, an analysis was done after exclusion of the reports from the US since most reports were retrieved from the US (84.3% of the reports for biologicals and 62.8% of the reports in the reference group were from the US). Third, an analysis was done in which reports from countries that only report ADRs involving small molecules were excluded from the reference group. The third analysis showed that only 0.7% of the suspected ADRs reported for the reference group were reported by countries that had not reported for biologicals. This is, therefore, expected to have a very limited effect on the results found, and the sensitivity analysis without these reports was not done. PRRs were also calculated to compare the different mechanistic classes. Within this analysis the mechanistic class hormones was used as the reference.

All statistical analyses were done using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). All analyses were unadjusted since this study was descriptive and not etiological in nature.

Table 1: A two-by-two table for a drug-suspected adverse drug reaction (ADR) combination in spontaneously reported data.

|	Suspected ADR of interest	All other suspected ADRs	Total
Biologicals	a	b	a+b
All other drugs (reference group)	c	d	c+d
Total	a+c	b+d	a+b+c+d

Proportional reporting ratio = $[a/(a + b)]/[c/(c + d)]$.

Results

General characteristics of case reports

Between January 1995 and December 2008, 191,004 case reports containing 546,474 suspected ADRs were reported for 62 different active biological substances. A total of 2,556,209 case reports containing 8,761,522 suspected ADRs were reported for all other drugs (reference group). From this data it can be estimated that 7.0% of the case reports reported to VigiBase concerned biologicals. The mean age for patients treated with a biological was 35.9 years, whereas the mean age for patients treated with a drug from the reference group was 50.7 years. For patients treated with biologicals, 27.8% were male and 68.5% were female compared with 37.5% males and 55.9% females for the reference group. Information on sex was missing in 3.7% of the reports for biologicals and 6.6% of the reports for the reference group. A case report for a biological contained, on average, 2.86 suspected ADRs, and a case report for a drug included in the reference group (mostly including small molecule drugs) contained, on average, 3.43 suspected ADRs. Figure 1 shows the cumulative number of case reports reported for the biologicals and the reference group available in VigiBase. The line of best fit for both groups seems to approach linear, which suggests that there was a constant number of case reports reported to VigiBase over the years. For the biologicals it was found that approximately two-thirds of all suspected ADRs were reported for five active substances: etanercept (20.3%), interferon-β-1a (15.6%), infliximab (11.6%), teriparatide (10.7%) and adalimumab (9.0%).

Figure 1: Cumulative number (%) of case reports reported for biologicals and the reference group.
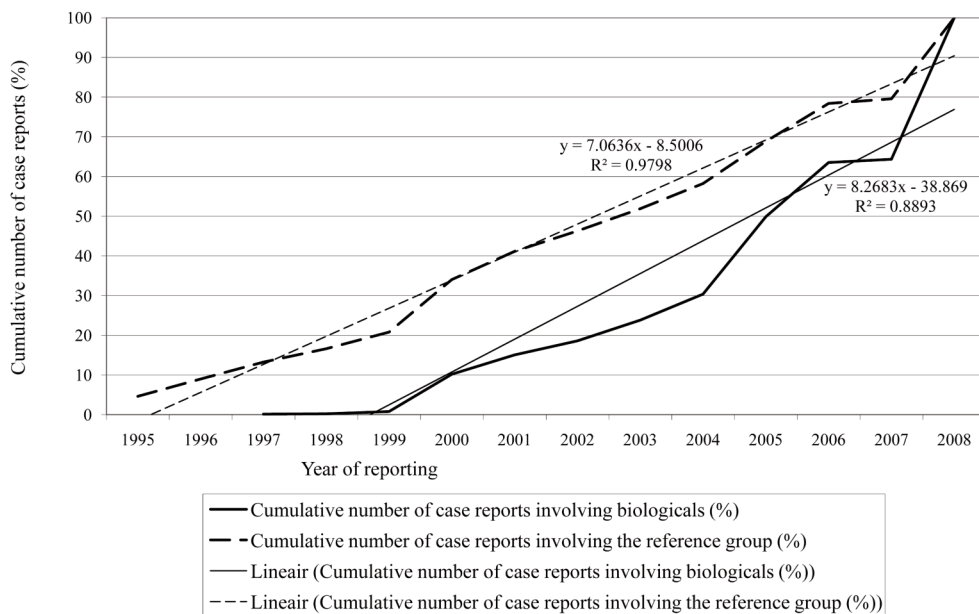
R^2 = square of the correlation coefficient

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Figure 2: Comparison of suspected adverse drug reactions reported for biologicals and the reference group at the System Organ Class level according to the Medical Dictionary for Regulatory Activities (MedDRA® version 12.0 (proportional reporting ratios [PRRs]). Vertical bars on the point estimates represent 95% CIs. PRR >1: more frequently reported for biologicals; PRR <1: more frequently reported for the reference group.
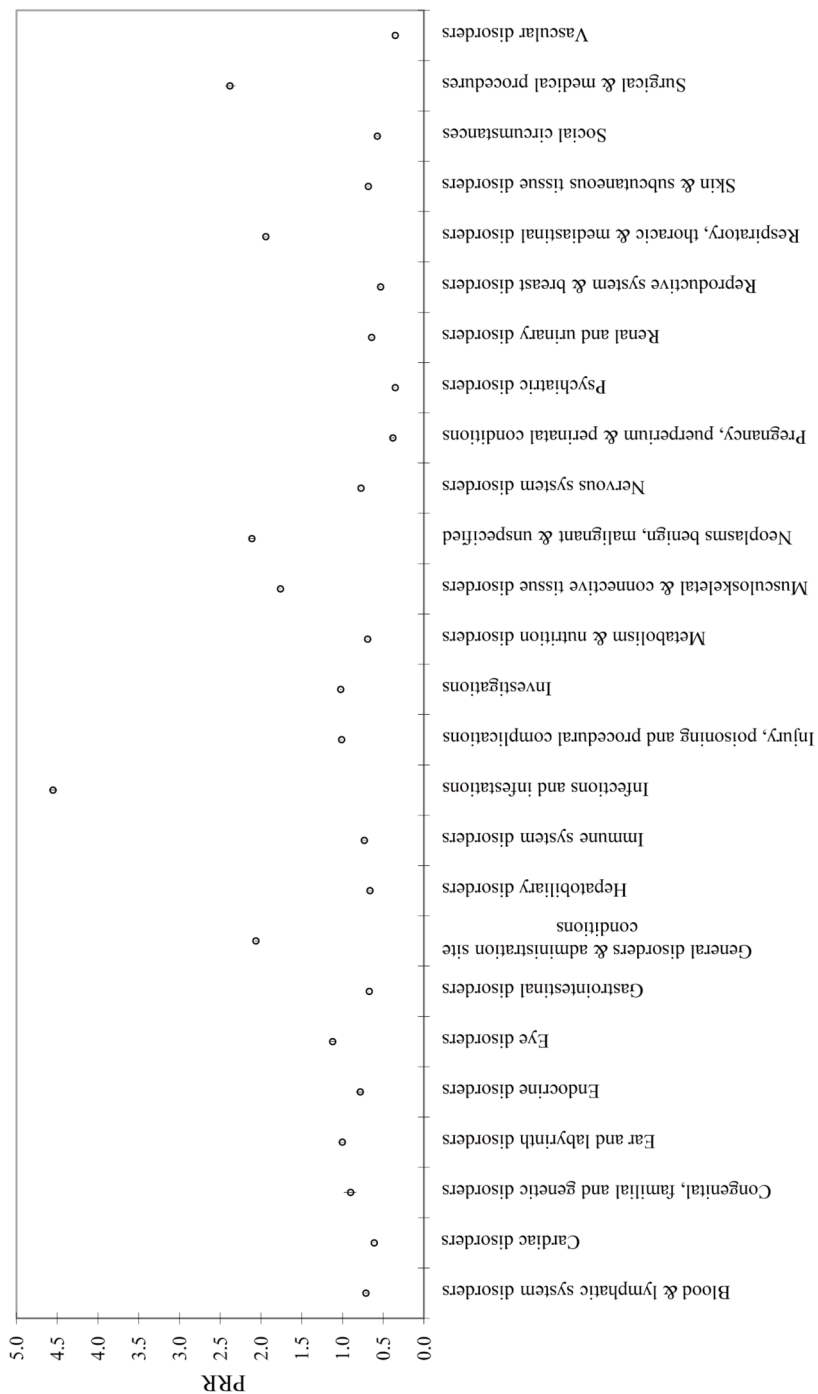
Figure 3: Comparison of suspected adverse drug reactions reported for biologicals, without the five biologicals most frequently implicated, and the reference group at the System Organ Class level according to the Medical Dictionary for Regulatory Activities (MedDRA® version 12.0 (proportional reporting ratios [PRRs]). Vertical bars on the point estimates represent 95% CIs. PRR >1: more frequently reported for biologicals; PRR <1: more frequently reported for the reference group.
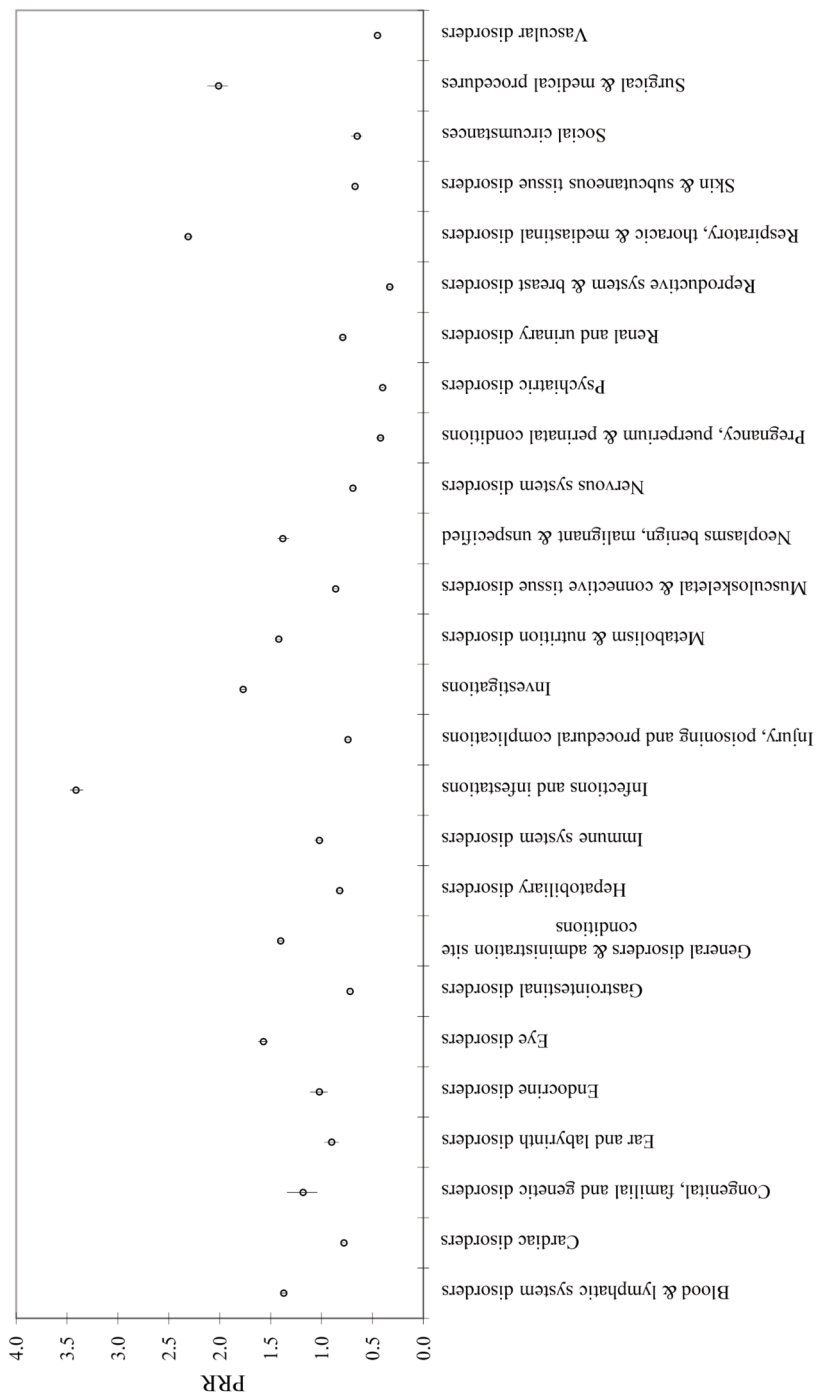
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Nature of suspected adverse drug reactions for biologicals versus the reference group

A comparison of the nature of the suspected ADRs reported for biologicals and the reference group showed clear differences between the suspected ADRs reported (Figure 2). Suspected ADRs for biologicals were more frequently reported in the SOCs 'Infections and infestations' (PRR 4.5), 'Surgical and medical procedures' (PRR 2.4), 'Neoplasms benign, malignant and unspecified' (PRR 2.1), 'General disorders and administration site conditions' (PRR 2.1) and 'Respiratory, thoracic and mediastinal disorders' (PRR 1.9) than for the reference group. Suspected ADRs in the SOCs 'Psychiatric disorders' (PRR 0.4), 'Vascular disorders' (PRR 0.4), 'Pregnancy, puerperium and perinatal conditions' (PRR 0.4), 'Reproductive system and breast disorders' (PRR 0.5) and 'Social circumstances' (PRR 0.6) were more frequently reported for the reference group than for biologicals. The analysis was also conducted without the five biologicals most frequently implicated to evaluate their effect on the calculated PRRs (Figure 3). Without these five biologicals, 57,282 case reports remained, including 178,595 suspected ADRs, and it was found that the SOCs 'Neoplasms benign, malignant and unspecified' and 'General disorders and administration site conditions' were no longer among the five SOCs in which suspected ADRs for biologicals were most frequently reported. These were replaced by 'Investigations' (PRR 1.7) and 'Eye disorders' (PRR 1.6). Certain SOCs were more frequently reported for biologicals with the five biologicals included and less frequently without, and *vice versa*. This includes the SOCs 'Musculoskeletal and connective tissue disorders' (PRR from 1.8 with the five biologicals most frequently implicated included to 0.9 without these five biologicals), 'Blood and lymphatic system disorders' (PRR from 0.7 to 1.4) and 'Metabolism and nutrition disorders' (PRR from 0.7 to 1.4).

The analysis without the reports from the US did not have a major impact on the results. Only for the SOC 'Injury, poisoning, and procedural complications' the PRR changed from 1.0 (US reports included) to 0.5 (US reports excluded), and for the SOC 'Neoplasms benign, malignant and unspecified' the PRR changed from 2.1 with US reports to 8.7 without US reports.

Safety profile of biologicals further elucidated and mechanistic classes compared

The safety profile of the biologicals was studied in more detail by stratification by mechanistic classes, with specific interest for the SOCs mentioned previously. However, because of the small percentage (0.9% of the total number) of suspected ADRs reported in the SOC 'Immune system disorders', this SOC was not studied in more detail.

'Infections and infestations' involved 8.7% of the total number of suspected ADRs reported for biologicals. Stratification by mechanistic class showed that more than 10% of the suspected ADRs reported for antibodies and receptors involved the SOC 'Infections and infestations' whereas these were less frequently reported for enzymes (4.7%) and hormones (3.1%) (Table 2). Compared with the mechanistic class hormones it was shown that 'Infections and infestations' were more frequently reported for all other mechanistic classes (Table 3). Further classification of suspected ADRs reported in this SOC into opportunistic and other infections showed that for all biologicals, about 16.9% of the reported infections could be classified as opportunistic infections. Opportunistic infections were mainly reported for antibodies (22.7%) and cytokines (23.3%).

Table 2: Distribution of reported suspected adverse drug reactions (ADRs) classified at the System Organ Class level for the total cohort of biologicals and stratified by mechanistic classes (%)[a].

System Organ Class[b]	All biologicals	Antibodies	Cytokines	Enzymes	Growth factors	Hormones	Interferons	Receptors
Infections and infestations	8.7	10.9	7.9	4.7	8.4	3.1	6.7	12.4
Neoplasms benign, malignant and unspecified	2.3	3.1	2.4	0.4	0.5	1.1	2.7	2.1
General disorders and administration site conditions	20.8	17.6	24.4	16.1	27.8	21.2	17.5	29.1
Nervous system disorders	9.5	7.3	5.9	11.5	7.4	10.1	15.4	7.1
Investigations	7.4	7.4	7.7	13.2	1.9	10.9	6.9	4.0
Gastrointestinal disorders	7.2	8.2	6.2	7.2	6.2	7.3	6.3	6.3
Other	44.1	45.5	45.5	46.9	47.8	46.3	44.5	39.0

a) Percentages are calculated based on the total number of suspected ADRs reported for biologicals and the individual mechanistic classes.
b) Suspected ADRs classified according to the Medical Dictionary for Regulatory Activities (MedDRA®) version 12.0.

Table 3: Safety profile of mechanistic classes compared [proportional reporting ratio (95% CI)][a].

Mechanistic class	Infections and infestations	Neoplasms benign, malignant and unspecified	General disorders and administration site conditions	Nervous system disorders	Investigations	Gastrointestinal disorders	Others
Hormones	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]
Antibodies	3.8 (3.7-3.9)	2.8 (2.6-3.0)	0.8 (0.8-0.8)	0.7 (0.7-0.7)	0.7 (0.6-0.7)	1.1 (1.1-1.2)	1.0 (1.0-1.0)
Cytokines	2.6 (2.4-3.0)	2.2 (1.8-2.6)	1.2 (1.1-1.3)	0.6 (0.5-0.6)	0.7 (0.6-0.8)	0.8 (0.7-0.9)	1.0 (1.0-1.1)
Enzymes	1.5 (1.4-1.7)	0.3 (0.3-0.5)	0.7 (0.7-0.7)	1.2 (1.1-1.2)	1.2 (1.2-1.3)	1.0 (0.9-1.1)	1.0 (1.0-1.1)
Growth factors	2.8 (2.2-3.5)	0.4 (0.2-1.1)	1.4 (1.2-1.6)	0.7 (0.6-0.9)	0.2 (0.1-0.3)	0.8 (0.7-1.1)	1.1 (0.9-1.2)
Interferons	2.2 (2.1-2.3)	2.4 (2.3-2.6)	0.8 (0.8-0.8)	1.6 (1.6-1.7)	0.6 (0.6-0.6)	0.8 (0.8-0.9)	0.9 (0.9-0.9)
Receptors	4.4 (4.2-4.6)	1.9 (1.7-2.0)	1.5 (1.5-1.6)	0.7 (0.7-0.7)	0.3 (0.3-0.4)	0.9 (0.8-0.9)	0.7 (0.7-0.7)

a) Suspected adverse drug reactions classified according to the Medical Dictionary for Regulatory Activities (MedDRA®) version 12.0.

Neoplasms benign, malignant and unspecified' involved 2.3% of the total number of suspected ADRs reported for biologicals. Of the suspected ADRs reported for antibodies, 3.1% involved this class whereas neoplasms were less frequently reported for enzymes, growth factors and hormones (Table 2). This is also reflected in the calculated PRRs where a significantly higher PRR was calculated for antibodies, cytokines, interferons and receptors, and a significantly lower PRR for enzymes as compared with the reference group (hormones) (Table 3). Subclassification of the suspected ADRs reported in this class showed that about 20% of the reported neoplasms for all biologicals involved haematological malignancies, 70% involved solid malignant tumours and about 10% were classified as other neoplasms. Differences between mechanistic classes were observed in the haematological malignancies and other neoplasms. Haematological malignancies were mainly reported for antibodies and cytokines, whereas for growth factors, two of five of the neoplasms reported involved mostly benign tumours.

'General disorders and administration site conditions' involved 20.8% of the suspected ADRs reported for all biologicals, whereas 29.1% of the suspected ADRs reported for receptors involved this SOC (Table 2). Classification of the reported immunological events in definite immunological events and possible immunological events showed that 17.0% of the suspected immunological ADRs could be classified as definite immunological and 83.0% as possible immunological.

Discussion

This study of the spontaneously reported suspected ADRs for biologicals in the WHO Global ICSR database, VigiBase, showed that approximately 7.0% of all case reports reported to VigiBase in the period from January 1995 to December 2008 concerned biologicals (vaccines were excluded from the analysis). Patients treated with biologicals for whom a suspected ADR was reported were, in general, younger than patients treated with the drugs in the reference group. This might have influenced the nature of the reported events for instance because older patients are more likely to develop various diseases related to their age. On the other hand, biologicals are often used to treat severe and/or life-threatening diseases,[1] which might have shifted the reporting of suspected ADRs to more serious events influencing the nature of the reported suspected ADRs.

In the present study, approximately two-thirds of all suspected ADRs were reported for five biologicals: etanercept, interferon-β-1a, infliximab, teriparatide and adalimumab. Etanercept, infliximab and adalimumab were in the top ten biotech drugs with the highest revenues in 2008 and 2007,[24] and etanercept and infliximab were both in the top ten biotech drugs with the highest US sales in 2005 and 2006.[25] Although sales and revenues can only be considered to give an indication of the actual number of users, it is interesting to note that other biologicals that have been in the top ten selling biotech drugs for the last 4 years, e.g. bevacizumab, rituximab (which was approved in the same year as interferon-β-1a) and pegfilgrastim (which was approved in the same year as adalimumab) are not among the five most reported biologicals.[24,25] Teriparatide, on the contrary, was approved in the US and EU around the same time as adalimumab and is not included in the top ten selling biologicals but is included in the top five products for which most suspected ADRs were reported to VigiBase. Within this context one should take into consideration

the benefit-risk balance of the drug and the number of patients exposed and should not only rely on the number of suspected ADRs reported. In general, more serious ADRs are accepted for drugs used to treat serious and/ or life-threatening conditions than for drugs used to treat less serious conditions.

In our study, the nature of the suspected ADRs reported for biologicals differed from that of the reference group. For biologicals, suspected ADRs related to 'Infections', 'Surgical and medical procedures', 'Neoplasms', 'General disorders and administration site conditions', and 'Respiratory, thoracic and mediastinal disorders' were more frequently reported than for the reference group. It is known from previous studies that infections in connection with the use of biologicals with an immunosuppressive action are important complications to be identified and communicated to healthcare professionals in the post-marketing setting.[7,26,27]

Biologicals are mostly administered by the parenteral route of administration, which makes these drugs more likely to cause infusion-like reactions and difficulties with their administration. Suspected ADRs related to infusion-like reactions are mostly classified in the SOC 'General disorders and administration site conditions'. The more frequent reporting of suspected ADRs in the SOC 'Respiratory, thoracic and mediastinal disorders' might be due to non-specific symptoms resulting from an immunological process, such as dyspnoea, asthma and shortness of breath. For the reference group, suspected ADRs related to 'Psychiatric disorders', 'Vascular disorders', 'Pregnancy, puerperium and perinatal conditions', 'Reproductive system and breast disorders', and 'Social circumstances' were more frequently reported. The low PRR calculated for the SOC 'Psychiatric disorders' could be explained by the reduced likelihood of biologicals to cross the blood-brain barrier due to their high molecular weight[28] limiting their effects on the CNS. Two of five of the SOCs in which suspected ADRs were more frequently reported for the reference group concerned suspected ADRs related to pregnancy and the reproductive system, which shows that ADRs related to pregnancy and the reproductive system are more frequently reported for the reference group. This might possibly be due to the reluctance of healthcare professionals to administer biologicals to pregnant women because of these agents being a relatively new class of drugs limiting their exposure and/or suspected ADRs related to pregnancy being more frequently reported for the reference group. A previous study, in which mostly small molecules were included, found that black-box warnings concerning risk in pregnancy were issued for 11% of the total number of black-box warnings issued. Risk in pregnancy was the fourth most frequent drug-related safety problem that triggered a black-box warning.[8] It was found that suspected ADRs for biologicals were more frequently reported in the SOC 'Surgical and medical procedures' and suspected ADRs for the reference group were more frequently reported in the SOC 'Social circumstances'. In this context it is important to note that the total number of suspected ADRs reported in these SOCs was less than 1.2% of the total number of ADRs reported, and it can be debated if events classified in these SOCs should be considered suspected ADRs. However, since the ADRs classified in these SOCs were considered to be related to the use of a specific biological or drug by the reporter it was decided to include the suspected ADRs reported in these SOCs in the study.

The SOCs in which suspected ADRs are most frequently reported for biologicals might include some important potential safety signals, which need to be studied in more depth during future studies. The example of tuberculosis with the use of infliximab[14] has already been discussed but it seems likely that there might be other specific safety signals for a biological or a group of biologicals within the SOC 'Infections and infestations'. The PRR of the SOC 'Neoplasms benign, malignant and unspecified' changed from 2.1 to 1.3 after the five biologicals most frequently implicated were not included in the dataset. This suggests that neoplasms are relatively frequently reported for (some of) these five active substances, which need to be elucidated further. A recent report referred to 121 case reports in VigiBase of leukaemia during the use of the tumour necrosis factor-α antagonists adalimumab, etanercept and infliximab,[29] which are all among the five biologicals most frequently implicated.

Our previous study showed that safety-related regulatory actions for biologicals were most frequently issued in the SOCs 'General disorders and administration site conditions', 'Infections and infestations', 'Immune system disorders' and 'Neoplasms benign, malignant and unspecified'.[7] The present study showed that suspected ADRs were most frequently reported in the SOC 'General disorders and administration site conditions' (20.8% of the total number of suspected ADRs reported for biologicals) (Table 2) and also that suspected ADRs in the SOC 'Infections and infestations' (8.7% of the total number of suspected ADRs reported for biologicals) were frequently reported. 'Neoplasms benign, malignant and unspecified' frequently triggered a safety-related regulatory action, but this SOC was only reported in 2.3% of the suspected ADRs reported for biologicals. However, a safety-related regulatory action is issued after a balanced assessment by the regulatory authorities and the need to inform healthcare professionals.[7] Because of their seriousness, safety issues related to neoplasms frequently trigger a safety-related regulatory action. In the current study, only 0.9% of the suspected ADRs were reported in the SOC 'Immune system disorders'. This might be explained by the reporting of less specific suspected ADRs, which are related to immunological events that are classified in different SOCs.

Results from the stratification of biologicals according to their mechanistic class suggest that pharmacovigilance could be targeted towards specific potential safety concerns for these subclasses, and these potential safety concerns should be specifically studied in the preregistration clinical trials, although the limitations of clinical trials should always be taken into account.[6] 'Infections and infestations' are, for example, frequently reported for antibodies and receptors. These findings might imply that infections should specifically be addressed in the pharmacovigilance plan of a new biological with an immunosuppressive mode of action, which often include biologicals classified in the mechanistic classes of antibodies and receptors. Due to the characteristic of biologicals that safety problems can often be related to an exaggerated pharmacology,[4] mode-of-action-driven safety assessment is important for biologicals and can lead to the prediction of potential safety problems. Many antibodies have, for example, an immunosuppressive effect, giving rise to infections.[30-32] A classification system for biologicals according to their pharmacology was recently proposed;[33] however, a more in depth classification of biologicals according to their specific pharmacology, e.g. depletion of B and/or T cells, seems to be relevant.

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This is important for the safety assessment of new biologicals to be approved in a specific class, but before this can be established, knowledge on how certain ADRs are related to the immunology of the human body should be obtained.

To our knowledge, this is the first study in which general characteristics of spontaneously reported suspected ADRs of biologicals are described in a broader perspective without the primary objective of finding potential safety signals for a specific active substance. This study, therefore, adds important information to the knowledge on the safety profile of biologicals. However, several potential limitations with this study need to be addressed. First, data was obtained from a spontaneous reporting system without additional causality assessment or qualitative verifications by the authors. Second, underreporting of suspected ADRs is a well recognised problem and is estimated to be in excess of 90%.[12] In addition, potential difficulties in the causality assessment of spontaneous reports of biologicals are expected. Biologicals are often indicated to treat severe and/or life-threatening diseases, patients treated with biologicals are often (pre)treated with multiple other drugs and/or suffering multiple other diseases,[1] and a relationship between intake of the drug and occurrence of the suspected ADR is often difficult to assess. Treatment with rituximab, for example, results in a depletion of B cells and it is known that it takes about 9–12 months before there is complete B-cell repletion.[34] Suspected ADRs occurring, for example, 6 months after treatment with rituximab is stopped might still be related to the previous treatment with the biological. In addition, at least 1.7% of the case reports in VigiBase were estimated to be duplicate reports,[35] which is especially a problem during signal detection. Since our study is descriptive in nature we feel that duplicate reports will not have a major impact on our results. Third, we found that about two-thirds of all suspected ADRs were reported for only five biologicals. This limits the generalisability of the results since these five biologicals have a large impact on the overall safety findings of the biologicals and it was shown that some results were altered after these five drugs were removed from the analysis. This should be taken into consideration during future analysis in VigiBase since these five drugs might distort the relation studied. This will mainly be a problem in case of quantitative signal detection and not necessarily in the event of the traditional case-by-case approach. Fourth, confounding by indication might have influenced the result. Biologicals are often indicated to treat serious and/or life-threatening diseases[1] and these patients might be more susceptible for the occurrence of suspected ADRs that are disease related. In addition, the nature of the suspected ADRs might also be influenced by the indication for which the drug was used, as shown by the high number of cases of multiple sclerosis (MS), which were mostly reported for interferon-β-1a. The cases of MS reported are likely to be due to disease progression or ineffectiveness of the administered drug instead of a suspected ADR. In addition, biologicals used to treat rheumatoid arthritis are, for example, related to the occurrence of infections. However, these patients already have an increased risk for infections.[16] This underlines the need to carefully think of a representative reference group during safety studies based on patient, disease and drug characteristics.

In this study, suspected ADRs were classified according to MedDRA® terminology. Recently, a novel classification of suspected ADRs for biologicals based on mechanistic considerations was proposed. Although the author states that the classification needs to

be evaluated in daily clinical care of patients,[22] this approach might improve signal detection and the ability to predict potential suspected ADRs for biologicals.

Conclusions

This study showed that in countries around the world the number of spontaneously reported suspected ADRs is increasing over time for all drugs, including biologicals, underlining the importance of these data sources for signal detection and hypothesis generation. However, during signal detection with biologicals in the WHO ADR database, VigiBase, one should be aware that five biologicals comprise two-thirds of the reported suspected ADRs, which might distort the relation found between a specific biological and a specific suspected ADR in the case of quantitative signal detection. Therefore, it is necessary to carefully consider the reference group to be used. In addition, causality assessment is expected to be complicated for biologicals.

Our study showed that the safety profile of biologicals and small molecules differed based on spontaneously reported suspected ADRs and that case reports of suspected ADRs to biologicals or classes of biologicals often refer to only a few SOCs, for instance infections with the use of immune suppressants (e.g. antibodies and receptors). This knowledge can be used in targeted preregistration clinical trials and in proactive pharmacovigilance activities to study particular safety issues and safeguard public health earlier and more effectively. In addition, since not all adverse reactions can be predicted or detected during development, spontaneous reporting remains an important tool for the early detection of signals of unexpected adverse reactions, interactions or other problems related to the use of biologicals.

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Adverse drug reactions of biologicals: how spontaneously reported adverse drug reactions fit a newly proposed classification system

Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Sabine M.J.M. Straus
Ronald H.B. Meijboom
Ivor R. Edwards
Hubert G.M. Leufkens
Toine C.G. Egberts

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Abstract

Background: Pichler has proposed a new classification system for adverse drug reactions (ADRs) of biologicals: high cytokine and cytokine release syndrome (type α), hypersensitivity reactions to biological agents (type β), immune/ cytokine imbalance syndromes (type γ), cross-reactivity (type δ), and non-immunological side-effects (type ε). One of the interests in pharmacovigilance is the identification of certain ADRs based on a combination of search terms. To be able to use the classification system as proposed by Pichler it is important to study if certain combinations of ADRs can be used for signal detection. The present study, therefore, aims to qualify and quantify how spontaneously reported ADRs fit the classification system as proposed by Pichler.

Methods: Spontaneously reported ADRs were selected from the WHO-ADR database VigiBase for cytokines, antibodies and fusion proteins and classified according to the classification system. No δ and only two α ADRs could be identified, and were therefore not included. In total, 17 type β, 21 type γ, and 24 type ε ADRs were included, for which Reporting Odds Ratios (ROR) were calculated (small molecules as reference). To study the correlation between pairs of ADRs cluster analysis and pair-wise dissimilarities were used.

Results: Cluster analysis resulted in 7 clusters; *cluster 1* contains 2 β and 22 ε ADRs, *clusters 2* contains 1 β, 13 γ, 2 ε, *cluster 3* contains 2 β and 4 γ ADRs, *cluster 4* contains 3 γ ADRs, and *clusters 5, 6, and 7* contain 5, 2, and 4 β ADRs, respectively. Calculated pair-wise dissimilarities for the ADRs classified according to Pichler showed differentiation between the type β and γ versus the type ε ADRs and correlation of ADRs related to the stage of the hypersensitivity reaction (infusion related relations, immediate-type hypersensitivity and delayed type hypersensitivity).

Conclusion: The proposed classification system seems to be valid for differentiation between the immunological β and γ ADRs and the non-immunological ε ADRs. Combination of ADRs related to type γ might also be useful for signal detection. Within the type β ADRs, related ADRs show correlation based on the phase of the hypersensitivity reaction.

Introduction

Biologicals are a relatively new class of drugs with specific characteristics as compared to the traditional chemically synthesised small molecule drugs, e.g. complicated production and purification process, high potential for immunogenicity, and limited predictability of pre-clinical to clinical data.[1] Although biologicals provide effective treatment options for a variety of chronic and serious conditions,[2] healthcare professionals should also take into account that biologicals have been linked to the occurrence of serious adverse drug reactions (ADRs).[3] A large share of these safety problems can be related to the pharmaceutical and mechanistic characteristics of these agents and mainly involve immunogenicity and immunosuppression.[1,4,5] The inherent immunogenic properties of biologicals can express in a variety of symptoms including Stevens-Johnson syndrome, angioedema and urticaria as well as decreased or even loss of efficacy due to the formation of neutralising antibodies.[5,6] Safety problems relating to immunosuppression are frequently expressed as infections and are often related to the mechanism of action, e.g. tuberculosis in patients using a tumour necrosis factor-alfa antagonist.[1,3,7,8]

Traditionally, ADRs of small molecules are often classified as either type A, type B, or type C ADRs.[9,10] Type A ADRs (drug actions) are related to the pharmacological activity of the drug, tend to be common, dosage-related and the risks can often be limited by using dosages that are appropriate for the individual patient. A typical example of a type A ADR is constipation during use of morphine.[10] Type B ADRs (patient reactions) on the other hand, are mainly allergic or idiosyncratic, only occur in a small minority of patients and are usually unexpected and unpredictable. Type B ADRs can comprise a variety of immunological reactions including anaphylaxis, vasculitis, and highly specific autoimmune syndromes. Type C ADRs (statistical effects) involves the increased occurrence of a given disease in patients using a particular drug, as compared with the (relatively high) background frequency in unexposed patients.[9,10] Based on the traditional classification system several other classification systems have been proposed. For example, Royer proposed in 1997 the use of four categories: type A (dose related), B (non-dose related), C (long term effects), and D (effects put later off).[11] Edwards and Aronson used the same definition for type A and B ADRs as Royer did but added type C (dose and time related), D (time related), E (withdrawal), and F (unexpected failure of therapy).[12] The most recent classification system is based on a three dimensional classification system based on dose relatedness, timing, and patient susceptibility.[13] None of these classification systems is specifically designed to classify ADRs of biologicals and the traditional classification of ADRs in type A, B, or C is still the most frequently used.

Compared to the traditional small molecule drugs, immunological reactions can be expected for biologicals based on their pharmacological properties. This is in contrast to the traditional definition of type B reactions and the concept of the traditional classification system of ADRs in type A and B ADRs for biologicals can, therefore, be questioned. In addition, ADRs related to immunogenicity and immunosuppression comprise an important part of the potential safety hazards related to the use of biologicals and further differentiation between these ADRs is expected to be valuable. Pichler therefore proposed a new classification system for ADRs of biologicals and more specifically for the biological agents belonging to the cytokines, antibodies and fusion proteins.[6] This new clas-

sification system classifies ADRs in five categories: high cytokine and cytokine release syndrome (type α), hypersensitivity reactions to biological agents (type β), immune/ cytokine imbalance syndromes (type γ), cross-reactivity (type δ), and non-immunological side-effects (type ε). According to Pichler, this new classification system might help to better understand and treat the patient who experiences ADRs during treatment with biologicals and might provide some help to avoid them in the future by defining risk factors and give directions for future research in this novel area.[6]

One of the interests in pharmacovigilance is the identification of certain ADRs based on a combination of search terms, such as in large spontaneous databases. A combination of known search terms related to, for example, type β ADRs will be important to identify hypersensitivity reactions as a potential problem with a biological agent. To be able to use the classification system as proposed by Pichler it is important to study if certain combinations of ADRs can be used for signal detection and generation and, if so, which combinations can be used. The present study, therefore, aims to qualify and quantify how spontaneously reported ADRs fit the classification system as proposed by Pichler.

Methods
Setting
Data were obtained from the International Drug Monitoring Program of the WHO. The WHO-adverse drug reactions (ADR) database, Vigibase, is maintained by the Uppsala Monitoring Centre and contains summaries of suspected spontaneous case reports originally summated by health care professionals and patients to national pharmacovigilance centers in 101 countries all over the world. As of October 2010, this database contained over 5.5 million case reports of suspected ADRs regarding specific, but anonymous, patients. The reports contain administrative data, patient data, ADR data, medication data and additional information. The information in these reports is not homogenous, at least with regard to origin, completeness of documentation or the likelihood that the suspected drug caused the adverse events.[15] ADR data in the reports are coded according to the Adverse Reaction Terminology (WHO-ART) and Medical Dictionary for Regulatory Activities (MedDRA).[16]

All suspected ADRs reported to VigiBase between January 1995 and December 2008 for the biological agents cytokines, antibodies and fusion proteins approved in the European Union and/ or the United States within this period were included, unless less than 100 ADRs were reported. This involved certolizumab pegol and technetium sulesomab. ADRs reported during this period for the small molecules were used as reference.

Classification of ADRs
Pichler proposed to classify ADRs of biologicals as type α (high cytokine and cytokine release syndrome), type β (hypersensitivity), type γ (immune (cytokine) imbalance syndrome), type δ (cross-reactivity), and type ε (non-immunological side effects) ADRs (Table 1).[6] From VigiBase the reported ADRs for the biologicals were listed according to their frequency of reporting. In each of the five categories the most frequently reported ADRs, with a maximum of 25 per category, were selected.

Table 1: Classification of adverse drug reactions according to Pichler[6] and selected ADRs included in the study.

	Short description	Selected ADRs
Type α ADRs	Connected to the systemic application of cytokines in relatively high doses or to high concentrations of cytokines released into the circulation	Cytokine release syndrome, cytokine storm
Type β ADRs	Three forms of allergy can be differentiated: IgE-, IgG- and T-cell mediated reactions	Anaphylactic reaction, anaphylactic shock, angioedema, antibody test positive, drug hypersensitivity, drug specific antibody present, hypersensitivity, injection site erythema, injection site rash, injection site reaction, injection site swelling, injection site urticaria, serum sickness, Stevens-Johnson syndrome, toxic epidermal necrolysis, type IV hypersensitivity reaction, urticaria
Type γ ADRs	Immunological events, but cannot be explained by high cytokine levels or typical hypersensitivity reactions. ADRs in this class can be further classified in ADRs related to an impaired function (immunodeficiency) and ADRs related to an immune or cytokine imbalance syndrome	Alveolitis allergic, arthritis bacterial, candidiasis, cytomegalovirus infection, herpes simplex, herpes zoster, histoplasmosis, lobar pneumonia, lupus-like syndrome, osteomyelitis, pharyngitis streptococcal, pneumocystis jiroveci pneumonia, pseudomonas infection, psoriasis, psoriatic arthropathy, pustular psoriasis, staphylococcal infection, staphylococcal sepsis, streptotoccal infection, systemic lupus erythematosus, tuberculosis
Type δ ADRs	Antibodies generated to an antigen expressed on tumour cells might also cross-react with normal cells, which express this structure as well, albeit to a lower degree	-
Type ε ADRs	Symptoms not directly related to the immune system	Ankle fracture, anxiety, blood glucose decreased, blood glucose increased, cardiac arrest, cardiac disorder, cardiac failure, cardiac failure congestive, convulsion, coronary artery disease, cerebrovascular accident, deep vein thrombosis, depression, diabetes mellitus, hip fracture, hyperglycaemia, hypoglycaemia, myocardial infarction, migraine, pulmonary embolism, thrombosis, transcient ischemic attack, weight decreased, weight increased

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The selected ADRs were independently classified as either type α, β, γ, δ, or ε by two re-searchers (TJG and AKM-T). ADRs not classified in the same ADR class were discussed with a third researcher (SMS) and if no agreement could be reached this ADR was ex-cluded from the analysis. Finally, two type α, 17 type β, 21 type γ, and 24 type ε ADRs could be included in the analysis. No ADRs specific for type δ ADRs could be selected (Table 1).

Analysis

Calculation of the reporting odds ratio

For each combination of a cytokine, antibody, or fusion protein with one of the selected ADRs a reporting odds ratio (ROR) was calculated as a measure of disproportionality. The ROR is the ratio of the reported odds of an ADR occurring in users of these agents (a/b) to the reported odds of it occurring in users of small molecule drugs (c/d) available in the database. The following formula was used: ROR = (a/b) / (c/d) = (a*d) / (b*c) (Table 2).[17] The ROR calculated for the combination tuberculosis – infliximab was for example 108.7 and means that tuberculosis is almost 109 times more frequently reported for infliximab compared to the reference group. A high ROR is highly suggestive for a relation between the use of infliximab and the occurrence of tuberculosis. In case a certain ADR was not reported, a cell in Table 2 obtained a zero and no ROR could be calculated. This was dealt with by adding 0.5 to every cell in Table 2[18] to be able to calculate an es-timate of the ROR.

RORs were calculated using SPSS version 16.0. For the analysis a $\log^{10}$ transformation of the ROR was used to limit the influence of very high RORs on the outcome.

Since only two ADRs could be classified as type α ADR and these ADRs were only re-ported for a very limited number of biologicals these were not taken into account in the analysis.

The calculated log RORs for the biological – selected ADR combinations was used in the cluster analysis and the calculation of the pair-wise dissimilarities which is described below in more detail.

Cluster analysis

Cluster analysis was used to identify clusters of the selected ADRs. Based on the calcu-lated log ROR, ADRs with a similar reporting profile cluster together in accordance with their overall homology. Related ADRs are considered to have a comparable reporting profile resulting in clusters of related ADRs. The length of the bars between the pair of ADRs reflects their dissimilarity; that is, the shorter the distance, the more closely related the pair of ADRs. Within the cluster analysis a maximum of ten clusters was considered. A heatmap was integrated in the dendrogram. A heatmap is a graphical representation of data in a two-dimensional map where the log ROR is represented by a spectrum of colours ranging from blue ($\log^{10} 0.01 = -2$) till red ($\log^{10} 888.37 = 2.95$).

Table 2: A two by two table for a drug (X)-adverse event (Y) combination in spontaneously reported data.

	Number of reports of ADR X	Number of reports with ADRs other than ADR X
Cytokine, antibody or fusion protein	a	b
Small molecules	c	d

Reporting Odds Ratio = (a/b) / (c/d) = (a*d) / (b*c)

Calculation of pair-wise dissimilarity

The cluster analysis provides a graphical representation of related ADRs based on their dissimilarity. Dissimilarities between pairs of ADRs can also be quantified. This is done by calculation of pair-wise dissimilarities, which is expressed as a number ranging between 0 and 2. Dissimilarity of "0" means that the pair of ADRs have perfectly correlated reporting profiles. Dissimilarity of "2" means that the pair of ADRs have perfectly anti-correlated reporting profiles. The expected dissimilarity of uncorrelated ADR profiles is "1". Within this study calculated pair-wise dissimilarities below 0.85 were considered to indicate related pairs of ADRs.

To quantify the results obtained with the cluster analysis, the average pair-wise dissimilarity was calculated for all combinations of ADRs in a cluster as well as the range and the percentage of calculated pair-wise dissimilarities below 0.85. These were also calculated for the combinations of ADRs as proposed by Pichler in type β, γ, and ε ADRs, and the sub-classification of type β ADRs in infusion related reactions, immediate-type hypersensitivity reactions, and delayed type hypersensitivity reactions. Cluster analysis and calculation of the pair-wise dissimilarities were performed with NCSS 2007.

Results

A total of 17 type β, 21 type γ, and 24 type ε ADRs reported for 28 cytokines, antibodies and fusion proteins were included in the analysis for which a total of 420,175 ADRs were reported (Table 3).

Cluster analysis and pair-wise dissimilarity

The cluster analysis with the integrated heatmap for the cytokines, antibodies and fusion proteins is shown in Figure 1. A row in the heatmap can be viewed as a barcode of the reporting profile of ADRs for the biologicals. By comparing these barcodes, clusters of ADRs based on the reporting profile can be identified and related ADRs are considered to have a comparable reporting profile resulting in clusters of related ADRs. In addition, the relatedness between pairs of ADRs can be quantified by calculation of the pair-wise dissimilarity, which results in a dissimilarity matrix.

From the cluster analysis seven clusters can be identified (Table 4). The type β ADR injection site reaction and the type γ ADRs cytomegalovirus infection and psoriasis were not clustered in any cluster.

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Table 3: Baseline characteristics of case reports reported for cytokines, antibodies, and fusion proteins and for the reference group[a].

|	Spontaneous reports for cytokines, antibodies, and fusion proteins (n=153,923)	Spontaneous reports for small molecule drugs (n=2,523,473)
Total number of ADRs in reports	420,175	8,625,435
Average number of ADRs in one report	2.73	3.42
Age of patient (mean (sd))	52.3 (16.3) years	50.6 (21.5) years
Female gender	68.8%	56.0%
Gender (missing)	3.5%	6.6%
Reporter		
Healthcare professional	38,4%	50.5%
Non-Healthcare professional	28.3%	10.8%
Literature	0.2%	0.2%
Unknown	15.1%	16.9%
Other	18.0%	21.5%
Region		
Europe	11.9%	30.5%
United States	84.0%	53.4%
Other	4.1%	16.0%

a) One case report represents one patient.

**Table 4: Results of cluster analysis in relation to calculated pair-wise dissimilarities.**

Cluster	ADRs in cluster	Average pair-wise dissimilarity (range)	Percentage of calculated pair-wise dissimilarities below 0.85
1	2 β, 22 ε	1.29 (0.60-1.74)	1.8%
2	1 β, 13 γ, 2 ε	1.09 (0.57-1.51)	12.5%
3	2 β, 4 γ	0.94 (0.65-1.17)	26.7%
4	3 γ	0.76 (0.63-1.01)	66.7%
5	5 β	1.09 (0.52-1.44)	20%
6	2 β	0.81[a]	100%
7	4 β	0.59 (0.46-0.66)	100%

a) A range is not presented since this cluster contains 2 ADRs, only one pair-wise dissimilarity can be calculated.

Figure 1: Dendrogram of cluster analysis and integrated heatmap of 62 adverse drug reactions for cytokines, antibodies, and fusion proteins.
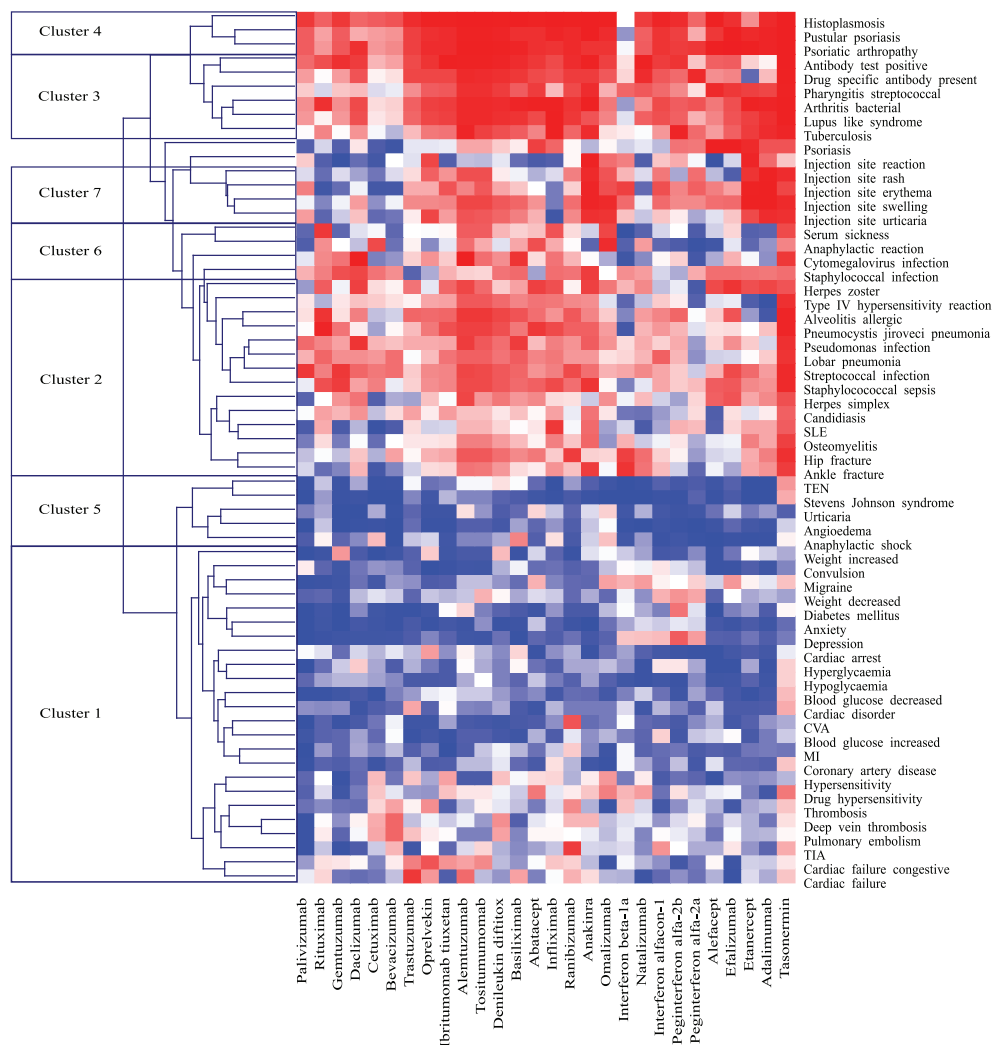
Logarithm of the reporting odds ratio
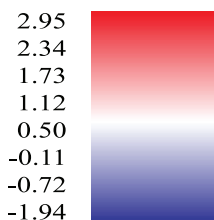
Chapter 3.2

Cluster 1 includes 22 of the 24 type ε ADRs and 2 type β ADRs. Within the dendrogram the two type β ADRs (hypersensitivity and drug hypersensitivity) are clustered together, which suggests correlation between these ADRs. Twenty-two of the 24 type ε ADRs are clustered together in the dendrogram suggesting a comparable reporting profile The calculated average pair-wise dissimilarity of 1.29 does, however, not suggest correlation of the ADRs within this cluster. This might be due to the large variety of ADRs classified as type ε ADRs.

Cluster 2 mainly contains γ ADRs and the average pair-wise dissimilarity of all combinations is 1.09. However, this cluster also contains one β and two ε ADRs. Based on the length of the bars between the pair of ADRs in the dendrogram it is shown that the related ADRs hip and ankle fractures are more similar compared to other combinations of ADRs in this cluster.

Cluster 3 consists of a mix of type β and γ ADRs. A closer look at the dendrogram shows that the type β ADRs are clustered together as well as the γ ADRs. The average pair-wise dissimilarity of 0.94 in this cluster suggests an uncorrelated ADR profile. However, 26.7% of the combinations of ADRs in this cluster have a pair-wise dissimilarity below 0.85.

Cluster 4 contains 3 type γ ADRs which is reflected in the dendrogram and the calculated average pair-wise dissimilarity of 0.76.

In *cluster 5*, 5 type β ADRs are clustered with an average pair-wise dissimilarity of 1.09. A closer look at this cluster shows two sub clusters. One cluster contains the highly related ADRs Stevens Johnson syndrome and toxic epidermal necrolysis with a pair-wise dissimilarity of 0.52 and the second sub cluster involves the ADRs urticaria, anaphylactic shock and angioedema with an average pair-wise dissimilarity of 0.97.

Cluster 6 contains the two β ADRs serum sickness and anaphylactic reaction.

Cluster 7 contains 4 of the 5 ADRs related to injection site reactions, which are highly correlated shown in the length of the bars between pairs of ADRs in the dendrogram and the calculated average pair-wise dissimilarity.

The proposed classification system by Pichler can be quantified in a dissimilarity matrix (Table 5). From the calculated pair-wise dissimilarities it is shown that the average pair-wise dissimilarity is 1.33 for all combinations of a type β-ε ADR and 1.34 for all combinations of a type γ-ε ADR. The average pair-wise dissimilarity suggests less correlation between the type β-ε ADRs and the type γ-ε ADRs compared to all possible combinations of type β-β ADRs (average: 1.18) and all combination of type γ-γ ADRs (average: 1.06). However, for all possible combinations of type β-γ ADRs an average pair-wise dissimilarity of 1.19 is calculated which is comparable to the combination of type β-β ADRs. About 15% of the calculated pair-wise dissimilarities for combinations of type β ADRs and for combinations of type γ ADRs is below 0.85, which suggests correlation between the pairs of ADRs.

Table 5: Dissimilarity matrix for combinations of selected ADRs.

Combinations of type β, γ, and ε ADRs according to Pichler

		Type β ADRs	Type γ ADRs	Type ε ADRs
Type β ADRs	Average dissimilarity (range)	1.18 (0.46-1.65)		
	% of dissimilarities <0.85	14.7%		
Type γ ADRs	Average dissimilarity (range)	1.19 (0.46-1.63)	1.06 (0.50-1.66)	
	% of dissimilarities <0.85	5.0%	16.2%	
Type ε ADRs	Average dissimilarity (range)	1.33 (0.69-1.66)	1.34 (0.71-1.77)	1.27 (0.60-1.74)
	% of dissimilarities <0.85	0.2%	0.8%	2.2%

Type β ADRs specified

		Infusion related reactions	Immediate-type hypersensitivity reactions	Delayed -type hypersensitivity reactions
Infusion related reactions[a]	Average dissimilarity (range)	0.67 (0.46-0.83)		
	% of dissimilarities <0.85	100%		
Immediate- type hypersensitivity[b]	Average dissimilarity (range)	1.37 (1.11-1.65)	0.93 (0.77-1.17)	
	% of dissimilarities <0.85	0%	33%	
Delayed type hypersensitivity[c]	Average dissimilarity (range)	1.38 (1.18-1.55)	1.19 (1.02-1.37)	0.74 (0.65-0.88)
	% of dissimilarities <0.85	0%	0%	66%

a) Injection site erythema, injection site rash, injection site reaction, injection site swelling, injection site urticaria.

b) Anaphylactic reaction, anaphylactic shock, angioedema, urticaria.

c) Antibody test positive, drug specific antibody present, type IV hypersensitivity reaction.

In addition, Pichler proposed to classify type β ADRs in immediate and delayed type hypersensitivity reactions (Table 5). From the dissimilarity matrix it is shown that the infusion-related reactions and the delayed-type hypersensitivity reactions have a correlated ADR profile. It is also shown that the combinations of infusion-related reactions with the immediate and delayed-type hypersensitivity reactions as well as the combination of immediate and delayed-type hypersensitivity reactions show no correlation suggesting that a clear differentiation can be made in the type β ADRs according to the stage of the hypersensitivity reaction.

Discussion

Immunotoxicity including immunosuppression and hypersensitivity (immunogenicity) are important potential safety problems of biologicals.[19,20] Pichler has proposed a new classification system of ADRs of biologicals, specific for the cytokines, antibodies, and fusion proteins.[6] This new classification system can be helpful for signal detection in, for example, large spontaneous databases in case certain related ADRs can be identified and combined, if they fit the classification system as proposed by Pichler. Within the present study we combined the results obtained with cluster analysis and calculated pair-wise dissimilarities. The validity of the method used for clustering of ADRs based on their re-porting profile is supported since related ADRs as hip and ankle fracture and Stevens-Johnson syndrome and toxic epidermal necrolysis were clustered in the same sub cluster and had a pair-wise dissimilarity close to 0.5.

In the ideal situation three clusters of ADRs would have been identified and the calculated pair-wise dissimilarities for the different combinations of the type β-β and type γ-γ ADRs would have, at least, all below 1. The type ε ADRs, however, consists of a wide variety of ADRs and an average pair-wise dissimilarity below 1 could therefore not be expected. Based on the calculated average pair-wise dissimilarities it is concluded that combinations of type β-β ADRs and combinations of type γ-γ ADRs are more correlated than combi-nations of type β-ε ADRs or combinations of type γ-ε ADRs. The cluster analysis also showed differentiation between the type β and γ ADRs on the one hand and the type ε ADRs on the other hand. This finding leads to the conclusion that, based on the reported ADRs, a distinction can be made between the immunological type β and γ ADRs and the non-immunological type ε ADRs. This distinction is less clear for the immunological type β and γ ADRs. The average pair-wise dissimilarity for all combinations of type β-γ ADRs was 1.19, which is comparable to the average pair-wise dissimilarity of 1.18 for the combination of type β-β ADRs. This was also reflected in the dendrogram in which 2 clusters contained a mix of type β and type γ ADRs. However, a closer look at the den-drogram showed that the type γ ADRs were mostly in two clusters, whereas the type β ADRs were mostly clustered in three sub clusters. The combination of the type γ ADRs in sub-cluster 2 can, therefore, be valuable for signal detection. Within the type β ADRs it can be concluded that clusters of correlated type β ADRs were formed based on overall homology and the phase of the hypersensitivity reactions, e.g. immediate hypersensitivity and delayed hypersensitivity. The differentiation of type β ADRs in immediate and de-layed was proposed by Pichler[6] and is supported by the findings of this study.

To our knowledge this study is one of the first to evaluate the classification system of ADRs as proposed by Pichler for pharmacovigilance purposes.[6] The strengths of this study are the use of ADRs that are mainly reported spontaneously by healthcare profes-sionals working in clinical practice and patients and the combination of the cluster analy-sis and the calculation of pair-wise dissimilarities. It is shown that large clusters (clusters with many ADRs) had more variety in the type of ADRs included. ADRs at the first and the last position in a large cluster are likely to be less related than ADRs clustered next to each other, which is also reflected in the calculated pair-wise dissimilarities. Combi-nation of these two methods leads to a more powerful study. However, this study deals with several assumptions and limitations. First, this evaluation is not complete since the

type α and δ ADRs could not be taken into account because ADRs specific for these groups were not frequently reported. The type α ADR cytokine storm consists of a variety of symptoms including headache, rigors, lumbar myalgia, hypotension, tachycardia, fever, respiratory distress and pulmonary infiltrates, renal impairment, and disseminated in-travascular coagulation as seen during the TeGenero phase I trial.[21] A patient presenting with a combination of these symptoms might result in the diagnosis of cytokine storm. However, in case cytokine storm as such is not reported but (some of) the symptoms are reported to the national pharmacovigilance centres, the case of cytokine storm would not have been identified resulting in misclassification and an underestimation of the actual number of cases of cytokine storm. The relatedness of certain ADRs was illustrated by Van Puijenbroek et al. In this study the authors found clustering of arthralgia, fever, and urticaria in spontaneous reports for terbinafine and concluded that these findings might point towards a clustering of these symptoms in patients using terbinafine suggesting a shared aetiology.[22] In this study, syndromes have not been taken into account and only ADRs which could be classified in one of the proposed ADR classes with some certainty were included. The value of our study would further increase with the identification of these so-called syndromes, which could be done as a next step in the evaluation of Pich-ler's proposed classification.

A second potential limitation with the use of spontaneous reports and the coding of ADRs according to MedDRA is the potential of misclassification and the classification of the same ADR with comparable ADR terms. In practice, a healthcare professional or a patient submits a case description to a national pharmacovigilance centre. In the national phar-macovigilance centre the reported ADR terms are coded to MedDRA with the potential for misclassification. We feel, however, that misclassification of reported ADR terms will not affect our results since we were not interested to study a relation between a certain biological and an ADR but to study the relation between different RORs. Assuming that misclassification is non-differential the relation between different RORs will not be largely affected. In theory, one ADR term for one patient can be classified according to two MedDRA terms, for example tachycardia and heart rate increased can both be re-ported for the same patient, and secondly cases were identified in which tachycardia was reported twice for the same patient. However, this involved less than 1% of the ADRs re-ported for biologicals and their influence on the final results is therefore expected to be limited.

Thirdly, we assumed pairs of ADRs to be correlated when the calculated pair-wise dis-similarity was below 0.85. This cut-off value was chosen based on the fact that a pair-wise dissimilarity of 0 means perfectly correlated ADR profiles where a pair-wise dissimilarity of 1 means uncorrelated ADRs. However, this cut-off value is not supported by data from literature, in which no cut-off values are described.

Fourthly, only the biologicals classified as cytokines, antibodies, and fusion proteins were taken into account as these were specifically described in the original paper by Pichler.[6] The other classes of biologicals including hormones and enzymes were not taken into ac-count in the present study since we first wanted to address the classes of biologicals specifically described by Pichler.

In conclusion, we found that for the cytokines, antibodies and fusion proteins the immunological type β and γ ADRs and the non-immunological type ε ADRs were clearly distinguished from each other. Two sub-clusters in which mainly type γ ADRs were clustered could be identified which could be useful for signal detection. Within the type β ADRs the ADRs that are closely related according to the stage of the hypersensitivity are in line with the classification as proposed by Pichler. This will be helpful in pharmacovigilance to combine certain ADR terms for signal detection in case one is for example interested in delayed type hypersensitivity reactions of biologicals. However, further research is needed for the ADRs classified as type α and δ since these could not be taken into account in the present study. The Drug Allergy and Hypersensitivity Database which was established by the European Network for Drug Allergy and the Global Allergy and Asthma European Network[23] might be a valuable data source to further evaluate the classification system as proposed by Pichler.

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Chapter 4

Risk factors and early warning markers in the clinical risk management of biologicals: rituximab as a learning case

Chapter 4.1

Invasive Aspergillosis in patients treated with rituximab for haematological malignancies

Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Sabine M.J.M. Straus
Andries C. Bloem
Tjomme van den Bruggen
Frits GmeligMeylin
Hubert G.M. Leufkens
Toine C.G. Egberts

Chapter 4.1

Abstract

Background: Invasive Aspergillosis (IA) is a major cause of death in oncology patients. Rituximab is indicated for several haematological malignancies and, although causality has not been established, two case reports and one epidemiological study have described a possible association of rituximab with IA. For invasive fungal disease it is known that early diagnosis and subsequent early initiation of therapy improves outcome.

Objectives: To estimate the incidence and identify risk factors for IA in patients treated with rituximab for haematological malignancies.

Methods: Patients treated with rituximab for haematological malignances between 2005 and 2008 within the University Medical Center Utrecht, the Netherlands, were included and a cumulative incidence was calculated. Within this cohort of patients a case-control analysis was performed, in which only the patients who had undergone allogeneic stem cell transplantation were included. The patients who developed IA were identified and classified as cases. For each case up to 3 controls were sampled. Potential risk factors were compared between cases and controls using a logistic regression model and presented as an odds ratio (OR) with the corresponding 95% confidence interval (95% CI).

Results: The cohort consisted of 104 rituximab treated patients of which seven patients were diagnosed with probable IA (cumulative incidence: 6.7%). The 104 patients had a median follow-up time of 9 months.

Patients who developed IA had been treated more frequently with a cumulative dose of rituximab of 1500 mg or more (OR 25.5; 95% CI 2.4-275.7) and had more frequently been diagnosed with another fungal infection in the 30 days before the diagnosis of IA (OR 15.0; 95% CI 1.2-183). All cases diagnosed with a fungal infection were diagnosed with a Candida infection.

Conclusion: The cumulative incidence seemed to be comparable to incidences found in other studies in patients with haematological malignancies. Patients treated with a high cumulative dose of rituximab and patients diagnosed with fungal infections in the 30 days before the diagnosis of IA were at an increased risk for IA.

Introduction

Invasive Aspergillosis (IA) is a major cause of death in oncology patients, especially after receipt of haematopoietic stem cell transplantation and therapy for haematological malignancies.[1-3] Neutrophils play an important role in the human defence against the Aspergillus species and prolonged neutropenia is a known risk factor for IA.[4,5] In addition, literature suggests that T-cells play a role in the human defence against IA and treatment with recognised T-cell immunosuppressants is a known risk factor for IA.[4-6] There may, however, also be a role for B-cells. Firstly, they produce antibodies, which may contribute to the defence against Aspergillus. Secondly, in the last decade it has been appreciated that B cells also play an important role as regulators of T cell reactivity. As professional antigen presenting cells they can activate T cells in vivo and by secretion of proinflammatory cytokines they can activate immune cells, including T cells, macrophages and natural killer cells.[7-9]

Rituximab is a chimeric anti-human monoclonal antibody which binds specifically to the transmembrane phosphoprotein CD20, which is expressed on pre-B and mature B-lymphocytes. Based on its mechanism of action rituximab is used in different types of haematological malignancies and auto-immune diseases. After binding to the CD20 antigen, rituximab induces lysis of target B-cells. Pre-registration studies have shown that peripheral B cell numbers rapidly decline below normal levels after administration of rituximab. Peripheral B-cell reconstitution starts within 6 months after treatment, reaching normal levels between 9 and 12 months after completion of therapy in patients treated with rituximab for haematological malignancies.[10-12] Treatment with rituximab in itself is not a known risk factor for the development of IA. Rituximab is, however, used in diseases in which the patients often undergo procedures and are treated with other drugs known to be related to IA. Based on the mode of action of rituximab, there might be a role in the human defence against IA. Two case reports and one epidemiological study have described cases of IA during and after treatment with rituximab. The first report described a case of primary hepatic IA after rituximab therapy for a post transplantation lymphoproliferative disorder[2] and a second report described a case of IA in a patient treated with rituximab for idiopathic thrombocytopenic purpura. The latter case had previously been treated with corticosteroids,[13] which is a known risk factor for IA.[4] The first report suggested that B-lymphocyte depletion induced by rituximab therapy probably increased the risk of IA,[2] whereas the second report did not specifically discuss the mode of action of rituximab in relation to IA.[13] In addition to these two case reports, a recent study evaluated the occurrence of IA following autologous haematopoetic stem cell transplantation. This study found that the cumulative incidence of IA was higher with the pre-transplant use (within 6 months before treatment) of rituximab (18.5%) versus no use of rituximab (4.9%) (HR 4.00; 95% CI 1.07-14.9).[14]

Early diagnosis of invasive fungal disease and subsequently early initiation of therapy improves outcome.[15,16] Therefore, within the present study we aim to estimate the incidence and identify risk factors for IA in a cohort of patients treated with rituximab for haematological malignancies using the Utrecht Patient Oriented Database (UPOD).

Chapter 4.1

Methods

Setting

The data was obtained from the Utrecht Patient Oriented Database (UPOD) of the UMC Utrecht (Utrecht, the Netherlands). The UMC Utrecht is a 1,042-bed academic teaching hospital in the center of the Netherlands, with approximately 28,000 clinical and 15,000 day-care hospitalisations and 334,000 outpatients annually. UPOD is a platform for clinical epidemiological research, the structure and content of which have been described in detail elsewhere.[17] In brief, UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders, and laboratory tests for all patients treated at the UMC Utrecht since 2004.[17] In addition, UPOD comprises a database with haematological data obtained with Cell-Dyn 4000 and Cell-Dyn Sapphire haematology analysers (Abbott Diagnostics, Santa Clara, CA, USA) used in routine blood cell analysis at the UMC Utrecht since January 2005. Per blood sample measurement all blood cell parameters the analyser is capable of measuring are automatically collected within the database, providing complete and validated automated haematological data, including absolute cell counts, cell volume indices, and morphological data.[18] UPOD data acquisition and data management is in accordance with current Dutch privacy and ethical regulation.

Design and study population

This study was designed as a cohort study. Initially, all patients treated with rituximab for haematological malignancies in the UMC Utrecht during the 4-year period between 1 January 2005 and 31 December 2008 were identified using electronic medication orders. Start of the first rituximab treatment was considered the index date and the rituximab treatment episode lasted up to 9 months after the last administration of rituximab. Patients could receive multiple administrations with rituximab resulting in a treatment episode which is longer than 9 months. A new treatment episode was considered in case of a gap between consecutive administrations of more than 9 months. The 9 month time period is based on the fact that it takes about 9-12 months for B-cell counts to reach normal levels after completion of rituximab treatment.[10,11] Only the first treatment episode was included in the analyses. IA occurring within the rituximab treatment episode was the outcome of interest.

A case-control study was performed in all patients from the study cohort who had undergone allogeneic stem cell transplantation. Allogeneic stem cell transplantation is an important risk factor for IA and is used for the diagnosis of IA, as described below.[4] For every case three controls were sampled. To limit the effect of time dependent variables, controls were sampled in a way that the follow-up time was the same as for the cases, e.g. if the case developed IA 50 days after the first rituximab administration three controls were sampled and for the controls data was included up to 50 days after the first rituximab administration. The timing between the allogeneic stem cell transplantation and the end of the follow-up time was comparable between the cases and the controls.

Invasive Aspergillosis

Patients with IA documented as proven or probable according to standardised definitions from the European Organisation for Research and Treatment of Cancer were used as cases.[4] In short, the likelihood of the diagnosis of IA is based on the categories proven invasive fungal infection, probable invasive fungal infection and possible invasive fungal infection.

• Proven IA is defined as histologic demonstration of invasive hyphae or a positive culture from a normal sterile environment (e.g. pleural fluid).

• Probable IA is defined as the presence of a host factor criterion (e.g. recent history of neutropenia, receipt of an allogeneic stem cell transplant, and/ or prolonged use of corticosteroids), and a clinical criterion (e.g. signs on CT scan, tracheobronchitis, sinonasal infection and/ or disseminated candidiasis), and a mycological criterion (e.g. galactomannan antigen detected in plasma, serum, broncheoalveolar lavage fluid, or cerebral spinal fluid, mold in sputum, broncheoalveolar lavage fluid, bronchial brush or sinus aspirate samples).

• Possible IA is defined as at least one host factor criterion and one clinical criterion. A mycological criterion is not part of the diagnosis of possible IA.[4,5,19] Patients with possible IA were not included as cases in the present study.

Allogeneic stem cell transplantation was the host factor criterion used to identify IA in this study and was, therefore, not included as a potential risk factor.

Potential risk factors

Several articles have identified potential risk factors besides allogeneic stem cell transplantation for the occurrence of IA, including age at bone marrow transplantation, underlying disease, use of fludarabine, and duration of neutropenia.[1,4,14] Within this study the following potential risk factors for the occurrence of IA were studied: 1) patient characteristics, 2) disease characteristics, 3) treatment characteristics, 4) haematological parameters, and 5) microbiological data.

1) Patient characteristics: age at the start of the episode and gender.

2) Disease characteristics: indication for use of rituximab (lymphoid or myeloid malignancies).

3) Treatment characteristics: cumulative dose of rituximab administered, concomitant use of medicines with an immunosuppressive effect, treatment with granulocyte colony stimulating agents (G-CSF) (ATC: L03AA), treatment with a nucleoside analogue (ATC: J05AB, J05AF and J05AR) and/ or treatment with an antimycotic drug for systemic use (ATC: J02A).

Medicines with an immunosuppressive effect included: immunosuppressants (L04), intestinal anti-inflammatory agents (A07E), glucocorticoids (H02AB), methylprednisolone, combinations (H02BX), glucocorticoids for respiratory intake (R03AB), glucosamine (M01AX05), gold thiolmalate (M01CB01), auranofin (M01CB03), chloroquine (P01BA01), and hydroxychloroquine (P01BA02). For glucocorticoids the cumulative dose was taken into account. This was done by calculating the prednisolon

equivalent dose for every separate glucocorticoid. The cumulative dose used was 1 mg of prednisolon stands equal to 5 mg cortisol, 4 mg hydrocortison, 0.8 mg methylpred-nisolon and triamcinolon, 0.15 mg dexamethason, and 0.13 mg betamethason. In general, use of 0.3 mg/kg/day of prednisolon equivalents is used a host factor criterion.[4] We had no data on the bodyweight of the patients. Prednisolon was, therefore, classified as <750 mg or ≥750 mg administered. In addition, all administered chemotherapeutic regimens (ATC code: L01) were collected. All of the described agents administered within the 90 days before the diagnosis of IA or before the end of the sampling period for the controls were included, except for the prednisolon equivalents, G-CSF and the antimycotic agents. The prednisolon equivalents and G-CSF were studied within 6 weeks and the antimycotics for systemic use within 14 days before the end of the sampling period (Figure 1).

Figure 1: Classification of risk factors. The different potential risk factors are studied in relation to the diagnosis of IA (cases) or the end of the sampling period (controls), meaning that the first administration of rituximab can be before (top figure) or within (lower figure) the different time periods classified for the risk factors[a].
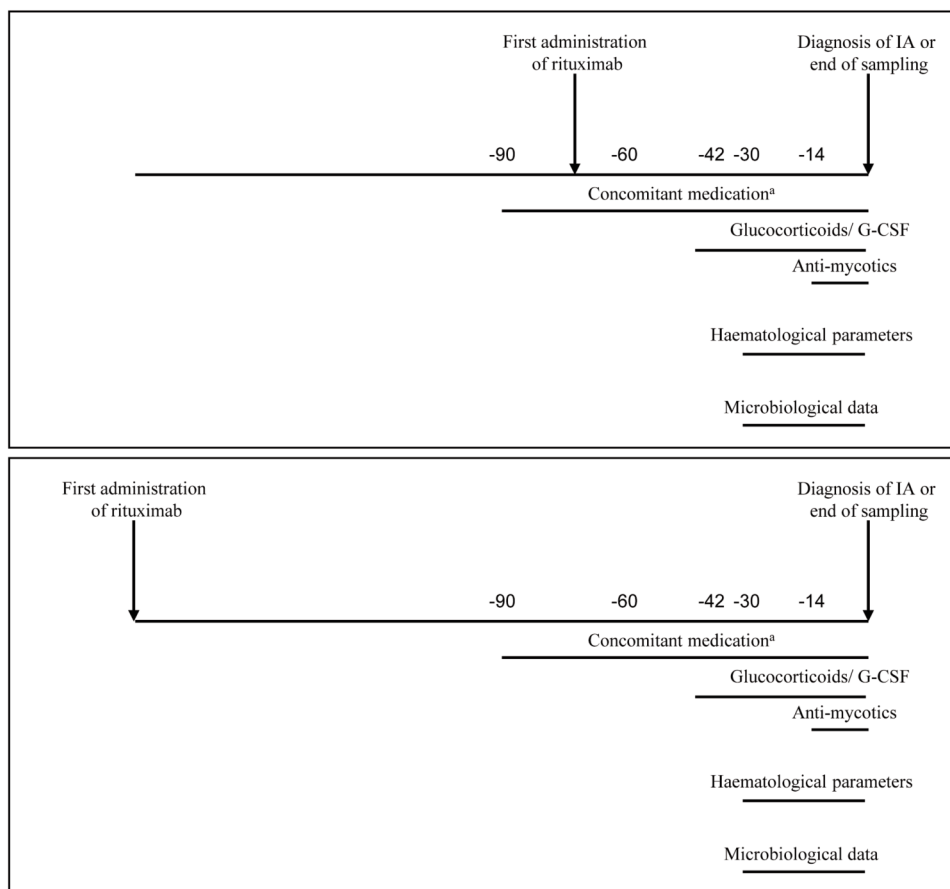
a) All drugs were studied within 90 days before the end of the sampling period, except for glucocorti-coids and G-CSF (both 42 days) and anti-mycotics (14 days).

4) Haematological parameters were classified as: grade 3 or 4 neutropenia (peripheral blood Absolute Neutrophil Count (ANC) $<0.5 \times 10^9$/L); grade 4 neutropenia (peripheral blood ANC $<0.2 \times 10^9$/L), grade 3 or 4 lymphopenia (peripheral lymphocyte blood count $<1 \times 10^9$/L), grade 4 lymphopenia (peripheral lymphocyte blood count $<0.5 \times 10^9$/L), and monocytopenia (peripheral monocyte blood count $<0.3 \times 10^9$/L). Episodes of the cytopenia within the 30 days before the end of the sampling period were constructed to determine the duration. A new episode was considered in case the patient had one or more measurements above the reference values or in case the patient did not have a blood measurement for longer than 2 weeks. The haematological parameters were classified based on the duration as <10 or ≥ 10 days.

5) Microbiological data: other fungal infections diagnosed by a positive culture within 30 days before the end of the sampling period were studied.

Data analysis

An estimate of the cumulative incidence was calculated and a Kaplan-Meier survival curve was constructed to graphically present the occurrence of IA in relation to the time after the first administration of rituximab. Within the total cohort of patients treated with rituximab and within the case-control analysis patients with and patients without IA were compared using a logistic-regression model and odds ratios (ORs) and corresponding 95% confidence intervals (CI) were presented. Due to the low number of cases a multivariate model was not applied.

All statistical tests were two-sided and a p-value smaller than 0.05 was considered statistically significant. All statistical analysis were done using SPSS 16.0 statistical software (SPSS Inc. Chicago, Illinois, USA).

Results

Between January 2005 and December 2008 a total of 104 patients have been treated with rituximab for haematological malignancies in the UMC Utrecht. The 104 patients had a median follow-up time of 9 months (range: 9-16 months) and 7 patients developed probable IA (cumulative incidence: 6.7%). No cases of definite IA were found. No significant differences were observed in age and gender between the patients with and without IA (Table 1). Patients with IA seemed to be diagnosed with myeloid malignancies more frequently (OR 5.1; 95% CI 0.8-31.5). However, this was not significant. All patients with IA had undergone allogeneic stem cell transplantation and 21 (21.6%) of the patients without IA had undergone this procedure.

The Kaplan-Meier curve showed that five cases were diagnosed within 6 months after the first administration of rituximab. The other two cases were diagnosed shortly thereafter (Figure 2). The median time to the diagnosis of IA after the first administration of rituximab was 90 days (range: 9-186 days). Based on the timing of the allogeneic stem cell transplantation for the patients with IA; five patients developed IA within 6 months after allogeneic stem cell transplantation, one patient developed IA 8 months after transplantation, and one patient developed IA 23 months after transplantation (Table 2).

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Table 1: General characteristics of patients with and without Invasive Aspergillosis compared within the cohort of rituximab users.

	Patients with IA (n=7)	Patients without IA (n=97)	OR (95% CI)
Patient characteristics			
Gender			
Male gender	6 (85.7%)	62 (63.9%)	1 [reference]
Female gender	1 (14.3%)	35 (36.1%)	0.3 (0.03-2.6)
Median age in years (range)[a]	42 (4-55)	53 (1-84)	1.0 (0.9-1.0)
Disease characteristics			
Diagnosis			
Lymphoid malignancies	5 (71.4%)	90 (92.8%)	1 [reference]
Myeloid malignancies	2 (28.6%)	7 (7.2%)	5.1 (0.8-31.5)
Allogeneic stem cell transplantation			
No	0 (0%)	76 (78.4%)	NA
Yes	7 (100%)	21 (21.6%)	

Figure 2: Kaplan-Meier curve for Invasive Aspergillosis after the start of treatment with rituximab.
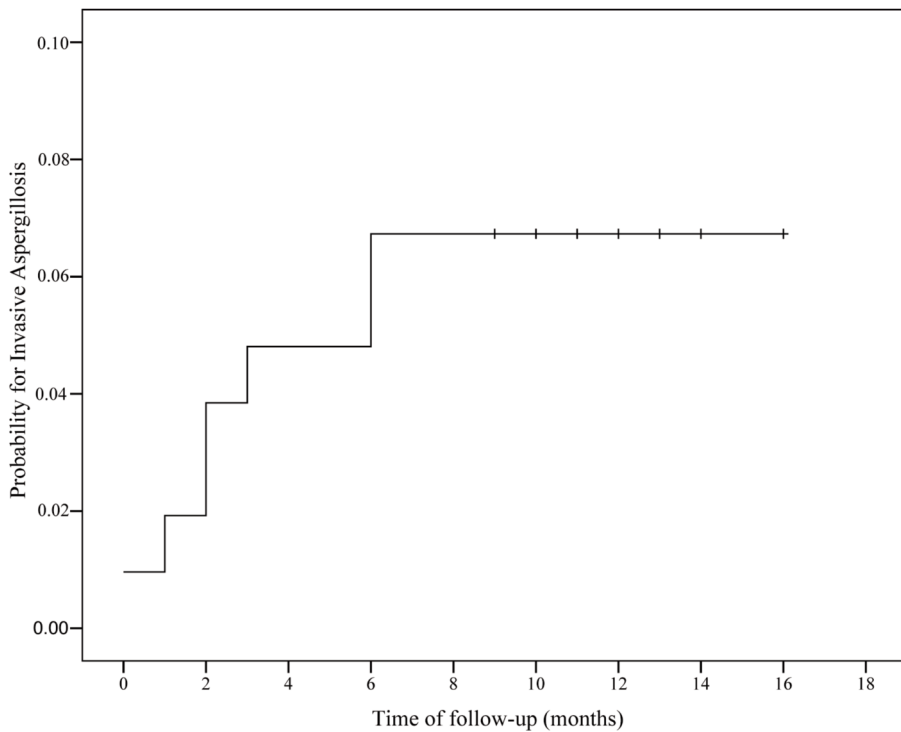
Patients with Invasive Aspergillosis

A description of the patients with IA is presented in Table 2. It is shown that only one of the seven patients with IA was female and three patients were aged below 18. Six patients were treated with an immunosuppressive agent and one patient received fludarabine within 90 days before the diagnosis of IA. Six patients were treated with nucleoside analogues and three patients were treated with antimycotic agents for systemic use within the 14 days before diagnosis of IA. However, two of these three patients were only treated with fluconazole which is not effective for the prevention/ treatment of IA. One patient was treated with fluconazole and intravenous amphotericin B. Intravenously administered amphotericin B can be helpful for the prophylaxis of IA. All three patients treated with antimycotic agents had been diagnosed with an infection with Candida species in the 30 days before the diagnosis of IA. Infection with Candida species were diagnosed by positive cultures from sputum or a nasal swab. This explains the treatment with fluconazole. Six patients had been treated with a cumulative dose of rituximab of 1500 mg or more of which two patients had received 2500 mg or more.

Risk factors for Invasive Aspergillosis

Within the patients who had undergone an allogeneic stem cell transplantation, patients with IA (cases) were compared to patients who did not develop IA (controls) (Table 3). Patients with and without IA did not differ with regard to age and gender. With regard to the treatment characteristics it was shown that cases had been treated with higher cumulative doses of rituximab at the time of diagnosis of IA. Treatment with a cumulative dose of rituximab of 1500 mg or more was related to a higher risk of IA (OR 25.5; 95% CI 2.4-275.7). The increased risk for IA was already present at a cumulative dose of rituximab administered of 1000 mg (OR 9.8; 95% CI 1.0-96.6). Although not statistically significant due to low numbers, patients treated with 750 mg or more prednisolon equivalents and patients treated with immunosuppressive agents, nucleoside analogues, and G-CSF seemed to be at an increased risk for IA.

A comparison of the haematological parameters showed that cases had lower lymphocyte and monocyte counts within the 30 days before the diagnosis of IA. However, this was not statistically significant. No difference between cases and controls was found with regard to the neutrophil count.

It was found that patients with IA had more frequently been diagnosed with one or more fungal infection (OR 15.0; 95% CI 1.2-183.6) within the 30 days before the diagnosis of IA. All fungal infections diagnosed concerned Candida species diagnosed by a positive culture from sputum or a nasal swab. As described previously three cases had been treated with anti-mycotics within the 14 days before the diagnosis of IA or before the end of the follow-up time. Four controls had been treated with anti-mycotics within this period. The anti-mycotic used involved fluconazole in three patients and itraconazol in one patient.

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Table 2: Patients with Invasive Aspergillosis.

Case	Gender	Age (yrs)	Indication	Time between diagnosis and ASCT (days)	Cum. dose rituximab (mg)	Cum. dose prednisolon (mg)	Immuno-suppressive agents	Nucleoside analogues	G-CSF	Antimycotic agents for systemic use	Cyto-tatics
1	M	55	NHL	132	2850	9077	Y	Y	Y	N	N
2	F	16	AML	246	1860	880	Y	Y	N	Fluconazol	N
3	M	10	AML	122	1575	792	Y	Y	N	N	N
4	M	4	ALL	82	735	1105	Y	N	Y	Fluconazol/ Amfotericine B	N
5	M	43	Multiple Myeoloma	727	2400	0	Y	Y	N	N	N
6	M	42	Burkitt lymphoma	20	3200	0	N	Y	N	N	Y
7	M	52	CLL	139	1500	4360	Y	Y	Y	Fluconazol	N

Case	Grade 3/4 neutropenia for ≥10 days	Grade 3/4 lymphopenia for ≥10 days	Monocytopenia for ≥10 days	Fungal infections
1	N	Y	Y	N
2	N	Y	N	Candida species
3	N	N	N	N
4	Y	Y	Y	Candida species
5	N	N	N	N
6	N	Y	Y	N
7	N	Y	Y	Candida species

F = Female; M = Male; ALL = Acute Lymphatic Leukaemia; AML = Acute Myeloid Leukaemia; CLL = Chronic Lymphatic Leukaemia; NHL = Non-Hodgkin Lymphoma; ASCT = Autologous stem cell transplantation; G-CSF = Granulocyt Colony Stimulating Factor.

Table 3: Characteristics of patients with Invasive Aspergillosis (cases) and patients with no Invasive Aspergillosis (controls).

	Cases (n=7)	Controls (n=21)	OR (95% CI)
Patient characteristics			
Gender			
Male gender	6 (85.7%)	15 (71.4%)	1 [reference]
Female gender	1 (14.3%)	6 (28.6%)	0.4 (0.04-4.2)
Median age in years (range)[a]	42 (4-55)	19 (1-65)	1.0 (1.0-1.1)[a]
Disease characteristics			
Diagnosis			
Lymphoid malignancies	5 (71.4%)	16 (76.2%)	1 [reference]
Myeloid malignancies	2 (28.6%)	5 (23.8%)	1.3 (0.2-8.8)
Treatment characteristics			
Cumulative dose of rituximab administered			
<1500 mg	1 (14.3%)	17 (81.0%)	1 [reference]
≥1500 mg	6 (85.7%)	4 (19.0%)	25.5 (2.4-275.7)
Cumulative dose of prednisolon administered within 6 weeks			
<750 mg	2 (28.6%)	15 (71.4%)	1 [reference]
≥750 mg	5 (71.4%)	6 (28.6%)	6.3 (0.9-41.5)
Use of immunosuppressive agents from the ATC class L04 within 90 days			
No	1 (14.3%)	10 (47.6%)	1 [reference]
Yes	6 (85.7%)	11 (52.4%)	5.5 (0.6-53.5)
Use of nucleoside analogues within 90 days			
No	1 (14.3%)	8 (38.1%)	1 [reference]
Yes	6 (85.7%)	13 (61.9%)	3.7 (0.4-36.6)
Use of G-CSF within 42 days			
No	5 (71.4%)	20 (95.2%)	1 [reference]
Yes	2 (28.6%)	1 (4.8%)	8.0 (0.6-107.0)
Use of cytostatic regimen within 90 days			
No	6 (85.7%)	19 (90.5%)	1 [reference]
Yes	1 (14.3%)	2 (9.5%)	1.6 (0.1-20.7)
Haematological parameters			
Grade 3 and 4 lymphopenia within 30 days			
<10 days	2 (28.6%)	16 (76.2%)	1 [reference]
≥10 days	5 (71.4%)	5 (23.8%)	4.1 (0.6-26.1)
Grade 4 lymphopenia within 30 days			
<10 days	4 (57.1%)	16 (76.2%)	1 [reference]
≥10 days	3 (42.9%)	5 (23.8%)	2.4 (0.4-14.6)
Grade 3 and 4 neutropenia within 30 days			
<10 days	6 (85.7%)	18 (85.7%)	1 [reference]
≥10 days	1 (14.3%)	3 (14.3%)	1.0 (0.1-11.5)
Grade 4 neutropenia within 30 days			
<10 days	6 (85.7%)	21 (100%)	
≥10 days	1 (14.3%)	0 (0%)	
Length of monocytes below 0.3×10^9/L within 30 days			
<10 days	3 (42.9%)	17 (81.0%)	1 [reference]
≥10 days	4 (57.1%)	4 (19.0%)	5.7 (0.9-38.1)
Microbiological parameters			
Fungal infections within 30 days			
No	4 (57.1%)	20 (95.2%)	1 [reference]
Yes	3 (42.9%)	1 (4.8%)	15.0 (1.2-183.6)

a) Not statistically significant.

Discussion

In patients treated with rituximab for haematological malignancies, we found a cumulative incidence of definite or probable IA of 6.7%. In the nested case-control analysis which was done in patients who had undergone an allogeneic stem cell transplantation, we identified treatment with a higher cumulative dose of rituximab and another fungal infection in the 30 days before the diagnosis of IA as risk factors for IA.

The cumulative incidence in this study is within the range of figures reported by others, although direct comparison is difficult due to differences with regard to patient characteristics, criteria used to diagnose IA, prophylactic use of anti-mycotics and so on.[1,14,20]

It was found in the case-control analysis that cases had been treated with higher cumulative doses of rituximab. This suggests a role of rituximab in the development of IA. Several studies have demonstrated a positive relation between serum rituximab concentrations and clinical response. In addition, it is known that a higher dose of rituximab administered increased the rituximab serum concentration and resulted in prolonged B-cell depletion. From the clinical trials it was shown that grade 3/4 lymphopenia occurred in about 40% of the patients treated.[21] Pre-clinical studies showed that recovery of B-cells occurred more rapidly in monkeys treated with lower doses of rituximab.[22] Although data on the relation between the dose of rituximab administered and the development of infections is limited, it seems likely that patients treated with higher doses of rituximab have more prolonged lymphopenia and are more susceptible for infections. An analysis of clinical trials in patients treated with rituximab in rheumatoid arthritis showed that patients treated with doses of 1000 mg had a non-significant increased risk (OR 7.20; 95% CI 0.43-120.66) for serious infections compared to patients treated with 500 mg rituximab.[23]

The role of rituximab in the development of IA has not been completely elucidated and is complicated due to patients being frequently treated with concomitant drugs with an immunosuppressive effect and suffering diseases and undergoing procedures known to be related to the development of IA, as was the case in our patients.[4] Within the present study we did not aim to study if patients treated with rituximab are at an increased risk for IA but to identify risk factors and to add to the understanding of the role of rituximab. As already described in the introduction, B-cells might also play a role in the human defence against IA. Although not statistically significant, it was found in the present study that patients who developed IA were more frequently diagnosed with grade 3/4 and grade 4 lymphopenia as compared to the patients who did not develop IA. Although no data were available on the differentiation between B- and T-lymphocytes this might be related to higher doses of rituximab administered, as described previously. Based on the higher doses of rituximab administered a role of rituximab in the development of IA seems likely. Confounding by indication might, however, have influenced the results and should be considered. Patients treated with higher doses of rituximab might, for example, be more diseased and therefore pose a higher risk for infections as compared to patients treated with lower doses of rituximab.

Cases were more frequently diagnosed with fungal infections as compared to the controls in the 30 days before the diagnosis of IA. All fungal infections diagnosed concerned Candida species and were treated with fluconazole. This finding showed that patients devel-

oping IA were already at an increased risk for other fungal infections, which probably relates to the immunosuppressed status of these patients.

Known risk factors for IA including treatment with an immunosuppressive agent, pred-nisolon equivalents, and nucleoside analogues were also identified in a cohort of ritux-imab users.[4] However, use of these agents was not significantly related to IA due to low numbers. Patients who have undergone allogeneic stem cell transplantation are often treated with these agents to prevent graft-vs.-host-disease and viral infections. Treatment with immunosuppressive agents can take up to several years after transplantation.[24,25] Neutropenia, which is also a known risk factor for IA,[4] was not identified as a risk factor for IA in the present study.

Within the present study, we identified treatment with a high cumulative dose of rituximab (1500 mg or more) and fungal infections in the 30 days before diagnosis of IA as risk factors for IA. These findings stress the importance of measures to minimise the risk for IA. The first step in the prevention of IA in high risk patients is the prevention of exposure to potentially invasive fungal species.[5,26] Patients should be educated to avoid circum-stances in which aerosols of fungal spores might be encountered, for example areas of high dust exposure, chicken coops, bat caves, and urban renewal and other construction projects. In addition, special efforts are needed to protect high risk patients from contact with air containing excessive fungal spore counts, including high efficiency particulate air (HEPA) filtration in patient rooms and high rates of room air exchange.[26] Preventive treatment with antifungal therapy can also be helpful in high risk patients, although lim-itations to mold-active prophylaxis exist due to the risk for drug interactions and adverse drug reactions but experience has been gained with mold-active azoles, echinocandins, and amphotericin B formulations.[5,26,27] Within the present study only one patient who de-veloped IA was treated with amphotericin B in the 14 days before the diagnosis of IA.

Within the present study we used data from UPOD which contains unique information collected during routine clinical practice.[17] Collection of data during clinical practice can add important knowledge about adverse drug reactions, which have not been identified during the clinical trials.[28] In addition, it was shown within the present study that these type of data sources can also be used to identify patients at risk for a certain adverse drug reaction. This study has several limitations which need to be addressed. Firstly, since UPOD is a hospital based registry, data from GP visits and use of drugs in the home setting might not have been complete. In addition, it might have been that patients died after discharge from hospital which could not be taken into account within the present study since this data was not available. However, most of the patients had a laboratory measurement or were treated with a drug shortly before the end of the treatment period. Secondly, since only a limited number of cases were identified we were not able to apply a multivariate model. The potential relation between two or more variables could therefore not be quantified.

In conclusion, within this study in patients treated with rituximab for haematological ma-lignancies we found a cumulative incidence for IA of 6.7%. Within the subpopulation of patients who had undergone allogeneic stem cell transplantation, patients treated with a high cumulative dose of rituximab and patients diagnosed with fungal infections in the

30 days before the diagnosis of IA were at an increased risk for IA. These findings stress the importance of measures to minimise the risk for IA, such as activities to minimise the exposure to potentially invasive fungus and preventive treatment with anti-fungal therapy.

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Chapter 4.2

**Rituximab-induced thrombocytopenia:
a cohort study**

Thijs J. Giezen
Aukje K. Mantel-Teeuwisse
Maarten J. ten Berg
Sabine M.J.M. Straus
Hubert G.M. Leufkens
Wouter W. van Solinge
Toine C.G. Egberts

Chapter 4.2

Abstract

Background: The combined information of drug exposure and laboratory test results on an individual patient level obtained in clinical practice can add important information about the safety of a drug. Thrombocytopenia is a known adverse drug reaction of rituximab but knowledge on risk factors and early warning markers is limited.

Objectives: To estimate the incidence and to explore risk factors and early warning markers for the development of rituximab- induced thrombocytopenia in clinical practice.

Methods: All patients treated with rituximab between 2005 and 2009 within the University Medical Center Utrecht, the Netherlands, were included in this study. The cumulative incidence for thrombocytopenia – defined as a platelet count below 100×10^9 platelets/L – was estimated within the 30 days after administration of rituximab. The frequency and magnitude of potential risk factors (patient, disease, treatment, and haematological laboratory characteristics before administration of rituximab) and early warning markers (haematological laboratory characteristics after administration of rituximab) were compared between rituximab exposed patients who developed (cases) or did not develop (controls) thrombocytopenia with a multivariate logistic regression model.

Results: Of the 90 included patients, 27 developed thrombocytopenia (cumulative incidence: 30%) within the 30 days after administration of rituximab and 18 patient developed grade 3/4 thrombocytopenia (cumulative incidence: 20%).
A multivariate model identified a relatively low platelet count (217.5 vs. 324.4×10^9 platelets/L; p=0.011) and, although not statistically significant, indication for use (OR 4.7; 95% CI 1.0-23.3) and a high platelet distribution width (PDW) (16.1 vs. 15.8; p=0.051), as independent risk factors for the development of thrombocytopenia before treatment with rituximab.

With regard to the potential early warning markers after administration of rituximab, patients developing thrombocytopenia had an average platelet count below 200×10^9 platelets/L in the week preceding the first measurement of thrombocytopenia whereas the average platelet count was above 200×10^9 platelets/L for patients who did not develop thrombocytopenia.

Conclusion: One out of three of the patients developed thrombocytopenia in the clinical setting after administration of rituximab, which was higher than identified during the clinical trials. Healthcare professionals should consider thrombocytopenia as a relevant ADR during treatment with rituximab. More frequent monitoring of the thrombocyte count is especially advised in patients treated with rituximab in the oncology indication and/ or with a relatively low platelet count and high PDW.

Introduction

Drug use is an increasingly common cause of isolated thrombocytopenia[1] and the estimated minimum incidence of such drug-induced thrombocytopenia (DIT) is about 10 cases per million inhabitants per year in the US and Europe.[2] Although DIT is uncommon it can have serious and sometimes even fatal consequences.[3] DIT is a known adverse drug reaction (ADR) of the monoclonal antibody rituximab, which is used for the treatment of Non-Hodgkin lymphoma, chronic lymphocytic leukaemia, and rheumatoid arthritis.[4,5] Clinical trials in which rituximab was administered as monotherapy for the treatment of relapsed low grade or follicular lymphoma reported grade 3/4 neutropenia, thrombocytopenia, and anaemia in 4.2%, 1.7%, and 1.1% of the patients, respectively.[5,6] It is known that differences exist between the clinical trial population and patients treated in clinical practice.[7] This might also result in higher incidences of observed ADRs. At this moment there is limited information on the incidence of rituximab-induced thrombocytopenia during use in clinical practice.

Identification of patients at increased risk for the development of DIT as well as early warning markers is important. More intensive monitoring of these patients may result in the identification of a drop in platelet counts at an earlier stage. Previous case reports have identified bone marrow involvement and splenomegaly as risk factors for rituximab-induced thrombocytopenia.[8,9] However, this is based on clinical observations of a limited number of patients and a comparison between patients with and without rituximab-induced thrombocytopenia has, to our knowledge, not been done in clinical practice.

The pathophysiology of DIT is diverse but can be divided in two major mechanisms: 1) decreased platelet production via marrow suppression and 2) increased peripheral platelet clearance, usually by an immune mechanism.[1] Antibody testing and bone marrow investigation can provide information on the underlying mechanism responsible for DIT. However, bone marrow investigation is invasive and often a burden for the patient, and antibody assays are not widely available. In addition, it may be difficult to obtain a sufficient number of platelets to perform laboratory tests with adequate sensitivity.[1,10,11] Haematological laboratory data have been used to explore the mechanism of DIT[11-13] and might be promising for use in clinical practice. The mechanism responsible for the development of thrombocytopenia following the administration of rituximab has not been completely elucidated and several potential mechanisms have been hypothesised including cytokine release syndrome post rituximab administration and an auto-immune mechanism. Based on these cases the hypothesised mechanisms for rituximab-induced thrombocytopenia are likely to be immune-mediated.[8,14,15]

Within the present study we aim to estimate the incidence and explore risk factors and early warning markers for the development of rituximab-induced thrombocytopenia in the clinical setting. Secondly, we aim to explore the underlying mechanism of rituximab-induced thrombocytopenia.

Methods

Setting

The data was obtained from the Utrecht Patient Oriented Database (UPOD) in the University Medical Center Utrecht (UMC Utrecht, Utrecht, the Netherlands). The UMC Utrecht is a 1,042-bed academic teaching hospital in the center of the Netherlands, with approximately 28,000 clinical and 15,000 day-care hospitalisations and 334,000 outpatients annually. UPOD is a platform for clinical epidemiological research, the structure and content of which have been described in detail elsewhere.[16] In brief, UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders, and laboratory tests for all patients treated at the UMC Utrecht since 2004.[16] Among other clinical data, UPOD comprises a database with haematological laboratory data obtained with Cell-Dyn 4000 and Cell-Dyn Sapphire haematology analysers (Abbott Diagnostics, Santa Clara, CA, USA) used in routine blood cell analysis at the UMC Utrecht since January 2005. Per blood sample measurement all blood cell parameters the analyser is capable of measuring are automatically collected within the database, providing complete and validated automated haematological data, including absolute cell counts, cell volume indices, and morphological data.[17] UPOD data acquisition and data management is in accordance with current Dutch privacy and ethical regulation.

Design and study population

This study was designed as a cohort study. Initially, all patients treated with rituximab in the UMC Utrecht during the 5-year period between 1 January 2005 and 31 December 2009 were identified using information from electronic medication orders. The day of start of the first rituximab treatment within this period was considered as the index date. A rituximab treatment episode lasted up to 30 days after the last administration of rituximab. In case of a gap between consecutive administrations of rituximab of more than 6 months this was considered a new treatment episode. Only the first treatment episode was included in the study. To be included in the study patients should have at least one complete blood count (including, among other parameters, haemoglobin, white blood cell counts, neutrophils, platelets, mean platelet volume [MPV], and platelet distribution width [PDW]) measured in the 30 days before the index date and one complete blood count within 30 days after a rituximab administration (event measurement) during the rituximab treatment episode. For patients with multiple blood counts before administration of rituximab, the measurement closest in time before the index date was selected. In addition, patients were excluded if: 1) patients obtained a transfusion with platelets within the 30 days before the start of the treatment episode and/ or before the event measurement, 2) patients had thrombocytopenia (platelet count less than 100×10^9 platelets/L) within the 30 days before administration of rituximab, and 3) patients were treated with rituximab for chronic idiopathic thrombocytopenic purpura independent of their platelet count measured before administration of rituximab. The remaining patients were included in the nested case-control analysis.

Thrombocytopenia

Thrombocytopenia was defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events as a platelet count below $100x10^9$ platelets/ L. In addition, the grade of thrombocytopenia, based on the platelet nadir, was classified according to these criteria: grade 1 ($75x10^9$/L- lower bound of the normal range), grade 2 (50-$74x10^9$/L), grade 3 (25-$49x10^9$/L), and grade 4 ($<25x10^9$/L).[18]

Risk factors and early warning markers for thrombocytopenia

Several patient, disease, treatment, and haematological characteristics were explored as potential risk factors. Patient characteristics included age and gender. Disease characteristic included the diagnosis for which rituximab was administered, classified as oncology and auto-immune diseases (patients treated for idiopathic thrombocytopenic purpura were excluded as described previously). Treatment characteristics were selected based on electronic medication orders and included the cumulative dose of rituximab administered, concomitant treatment with cytostatics, corticosteroids or other drugs known to be related to thrombocytopenia (Appendix 1).

The following haematological laboratory parameters were studied: haemoglobin, white blood cell counts, neutrophils (neutrophil granulocytes), platelets, MPV, and PDW. These were compared before administration of rituximab and during the rituximab treatment episode. The haematological parameters measured before administration of rituximab were classified as risk factors and after administration of rituximab these were classified as early warning markers for thrombocytopenia.

Exploring the mechanism of rituximab-induced thrombocytopenia

The mechanism underlying rituximab-induced thrombocytopenia was explored based on the following characteristics, which are used as proxy measures:

1) For all patients the values of the MPV and PDW for the baseline and the event measurement were identified. In general, immune-mediated thrombocytopenia results in activation of the bone marrow to increase the platelet production resulting in the release of younger, larger platelets with an increase in platelet size indices. Thrombocytopenia caused by bone marrow suppression on the other hand results in normal or even smaller platelet measures.[10,11,19] MPV is calculated by the haematology analyser as the arithmetic mean from the impedance platelet histogram and is reported in femtoliter (fL). Per patient it was determined whether the MPV was abnormally high, defined as >9.5 fL based on the upper limit of the reference range used at the UMC Utrecht. PDW is calculated by the haematology analyser from the impedance platelet histogram and is reported in ten times the geometric standard deviation (GSD).[20]

2) Thrombocytopenia after re-exposure to a drug is a marker of immune-mediated thrombocytopenia.[10] Electronic data on drug exposure was only available from January 2004 onwards. Before that period only data on diagnosis, coded by ICD-9-CM code (Appendix 2) was available. Based on the ICD-9-CM codes patients likely to have been treated with rituximab before 2004 were selected and for these patients the letter of the

specialist to the GP send during the study period was checked if mention was made of previous exposure to rituximab.

3) The timing between intake of rituximab and the occurrence of the first platelet measurement below 100×10^9 platelets/ L. In general, immune-mediated thrombocytopenia can develop at any moment while thrombocytopenia as the result of bone marrow suppression generally occurs two weeks or longer after administration of the drug.[1,10] In addition, in immune-mediated thrombocytopenia the decline in platelets is normally rapid whereas the number of platelets gradually decline during thrombocytopenia as a result of bone marrow suppression.[1,10]

4) Although there are exceptions, isolated thrombocytopenia commonly occurs in immune-mediated thrombocytopenia.[10] An event of isolated thrombocytopenia was defined as the presence of a platelet count less than 100×10^9/L without anaemia (haemoglobin > 9.7g/dL), leucopenia (leucocyte count > 4.0×10^9/L) and neutropenia (neutrophil granulocyte count > 1.6×10^9/L) based on the same complete blood count. Non-isolated thrombocytopenia was defined as a platelet count less than 100×10^9/L with concurrent anaemia (haemoglobin $\leq$ 9.7g/dL) and/ or leucopenia (leucocyte count $\leq 4.0 \times 10^9$/L) and/ or neutropenia (neutrophil granulocyte count $\leq 1.6 \times 10^9$/L) based on the same complete blood count.

Data analysis

An estimate of the cumulative incidence of rituximab-induced thrombocytopenia was calculated. A Kaplan-Meier survival curve, stratified by indication for use, was constructed to graphically represent the occurrence of thrombocytopenia in relation to the time after the administration of rituximab.

Within the nested case-control study patients with (cases) and patients without (controls) thrombocytopenia were compared regarding risk factors with a univariate unconditional logistic regression model and presented as odds ratios (OR) with 95% confidence intervals. For the haematological parameters only a p-value was presented. Since the haematological parameters were in general not normally distributed a median was presented in stead of a mean value. To test for potential confounders, two multivariate models were applied in which the potential risk factors and early warning markers with a $p < 0.1$ in the univariate model were included.

All statistical tests were two-sided and a p-value smaller than 0.05 was considered statistically significant. All statistical analysis were done using SPSS 16.0 statistical software (SPSS Inc. Chicago, Illinois, USA).

Results

Between January 2005 and December 2009 a total of 235 patients has been treated with rituximab in the UMC Utrecht, of which 90 patients (38.3%) were eligible for inclusion (Figure 1). Twenty-seven patients developed thrombocytopenia after administration of rituximab (cumulative incidence: 30%) and 18 patients developed grade 3 or 4 thrombocytopenia (cumulative incidence: 20%) within 30 days after administration of rituximab. The mean platelet count at nadir in the patients who developed thrombocytopenia was 47×10^9

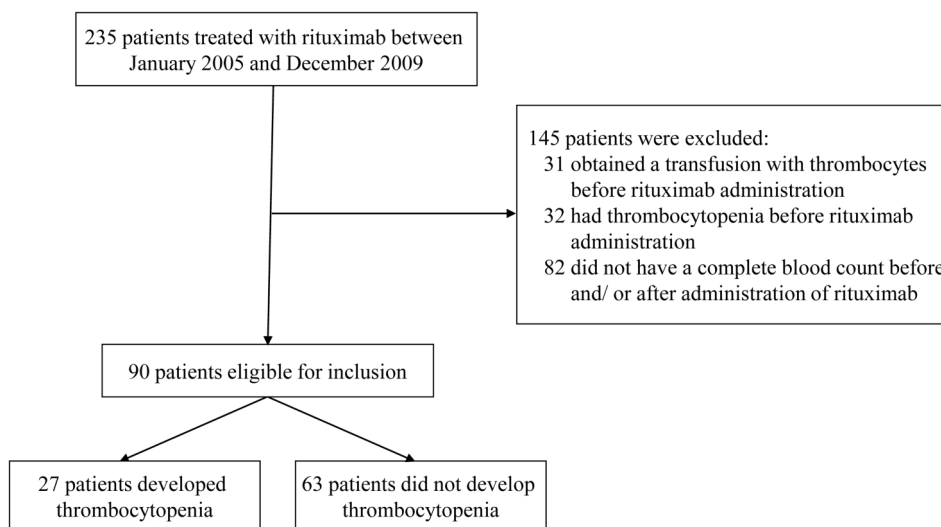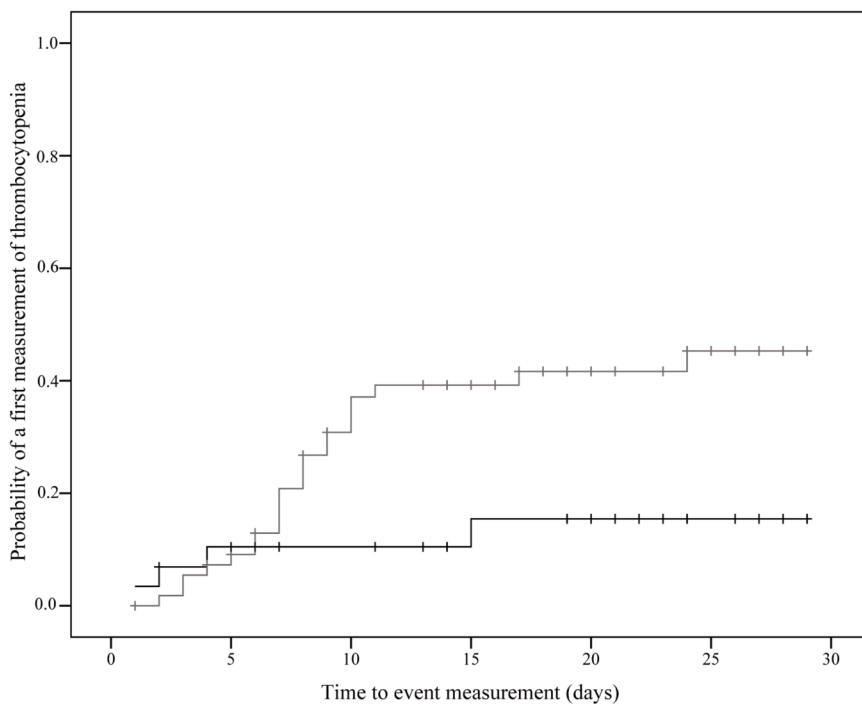**Figure 1: Patients included in the study.**

Figure 2: Kaplan-Meier curve for the first measurement of thrombocytopenia after administration of rituximab for the oncology indication (grey line) and for the auto-immune indications (black line).

platelets/L (range: 7-96x10^9 platelets/L). Kaplan-Meier survival analyses showed that most cases of thrombocytopenia occurred within 10 days after administration of rituximab for both groups of patients treated for oncology or autoimmune diseases (Figure 2).

Risk factors and early warning markers for thrombocytopenia

The baseline characteristics of patients that developed thrombocytopenia (cases) and patients that did not develop thrombocytopenia (controls) is shown in Table 1. Patients treated with rituximab in the oncology indication were at an increased risk for the development of thrombocytopenia as compared to patients treated with rituximab for an auto-immune disease (OR 4.9; 95% CI 1.5-15.8) (Table 1). Concomitant use of cytostatics was not associated with an increased risk for thrombocytopenia (OR 1.1; 95% CI 0.4-2.6). Concomitant treatment with any drug associated with thrombocytopenia was not identified as a risk factor for the development of thrombocytopenia. An analysis with the separate drugs known to cause thrombocytopenia identified patients concomitantly treated with ciprofloxacin (OR 6.5; 95% CI 2.3-18.2) or fluconazole (OR 4.4; 95% CI 1.6-11.9) as being at an increased risk for the development of thrombocytopenia. Use of corticosteroids within the 14 days before the first platelet count measurement below 100x10^9 platelets/L had no influence on the development of thrombocytopenia. The haematological parameters measured before administration of rituximab showed that patients who developed thrombocytopenia had a higher PDW (16.1 vs. 15.8; p=0.025) and a lower platelet count (217.5 vs. 324.4x10^9 platelets/L; p=0.015) compared to patients who did not develop thrombocytopenia. A multivariate model with all risk factors with a p-value below 0.1 in the univariate analysis identified a low platelet count (p=0.011) and, although not statistically significant, indication for use (OR 4.7; 95% CI 1.0-23.3) and a high PDW (p=0.051) as independent risk factors for thrombocytopenia.

Figure 3 shows the average platelet count, MPV, and PDW count after administration of rituximab in the week before the platelet count dropped below 100x10^9 platelets/L for the cases and before the lowest platelet count was reached for the controls. The platelet count was lower for the cases in the week before the diagnosis of thrombocytopenia. The cases all had an average absolute platelet count below 200x10^9 platelets/L in the week before the first measurement of thrombocytopenia. Although the average platelet count of the controls remained above 200x10^9 platelets/L in the week before their lowest platelet count was reached it should be noted that their average platelet count also started to drop from 4 days before the lowest platelet count was reached. The average MPV was higher for the cases from 4 days before the diagnosis of thrombocytopenia. The absolute difference between the two groups became more distinct at 1 day before the diagnosis of thrombocytopenia. The average PDW was higher for the cases and lower for the controls in the five days before the diagnosis of thrombocytopenia or before the lowest platelet count was reached. However, the absolute difference between both groups of patients was small.

Exploring the mechanism of rituximab-induced thrombocytopenia

Table 2 provides an overview of the patients developing thrombocytopenia. Overall, an immune-mediated mechanism might have played a role in 19 (patient numbers presented in bold in Table 2) of the 27 patients (70.4%) with thrombocytopenia and can not be excluded for the other 8 patients.

**Table 1: Identification of risk factors for thrombocytopenia.**

	Patients with thrombocy-topenia (n=27)	Patients with-out thrombo-cytopenia (n=63)	Odds Ratio (95% CI)	p-value
Patient characteristics				
Male	16 (59.3%)	38 (60.3%)	1 [reference]	0.925
Female	11 (40.7%)	25 (39.7%)	1.0 (0.4-2.6)	
Mean age in yrs (sd)	45.8 (20.5)	47.5 (23.2)	1.0 (1.0-1.0)	0.738
Indication for use of rituximab				
Auto-immune	4 (14.8%)	29 (46.0%)	1 [reference]	0.008
Oncology	23 (85.2%)	34 (54.0%)	4.9 (1.5-15.8)	
Treatment characteristics with rituximab				
<700 mg rituximab administered	3 (11.1%)	17 (27.0%)	1 [reference]	
≥700 and <1000 mg rituximab administered	16 (59.3%)	24 (38.1%)	3.8 (1.0-15.0)	0.059
≥1000 mg rituximab adminstered	8 (29.6%)	22 (34.9%)	2.1 (0.5-9.0)	0.335
Use of drugs associated with thrombocytopenia				
Use of cytostatics				
No	15 (55.6%)	36 (57.1%)	1 [reference]	0.889
Yes	12 (44.4%)	27 (42.9%)	1.1 (0.4-2.6)	
Use of drugs associated with thrombocytopenia				
No	3 (11.1%)	12 (19.0%)	1 [reference]	0.360
Yes	24 (88.9%)	51 (81.0%)	1.9 (0.5-7.3)	
Use of ciprofloxacin				
No	13 (48.1%)	54 (85.7%)	1 [reference]	0.000
Yes	14 (51.9%)	9 (14.3%)	6.5 (2.3-18.2)	
Use of fluconazol				
No	14 (51.9%)	52 (82.5%)	1 [reference]	0.004
Yes	13 (48.1%)	11 (17.5%)	4.4 (1.6-11.9)	
Use of corticosteroids within 14 days before platelet count below 100x10^9 platelets/L				
No	10 (37.0%)	23 (36.5%)	1 [reference]	0.962
Yes	17 (63.0%)	40 (63.5%)	1.0 (0.4-2.5)	
Haematological parameters				
Haemoglobin (g/dL)[a]	13.3	11.7	-	0.230
White blood cell count count (x10^9/L)	8.8	8.5	-	0.534
Mean platelet volume (fL)	7.8	7.4	-	0.724
Neutrophil count (x10^9/L)	5.6	6.1	-	0.932
Platelet distribution width (10GSD)	16.1	15.8	-	0.025
Platelet count (x10^9/L)	217.5	32	-	0.015

GSD = geometric standard deviation.

a) 15 patients had received a transfusion with red blood cells before the blood measurement and were therefore excluded from the analysis of haemoglobin level.

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Figure 3: Pattern of platelet count (a), mean platelet volume, (b) and platelet distribution width (c) from 7 days before the diagnosis of thrombocytopenia for patients developing (n=27) and patients not developing (n=63) thrombocytopenia.
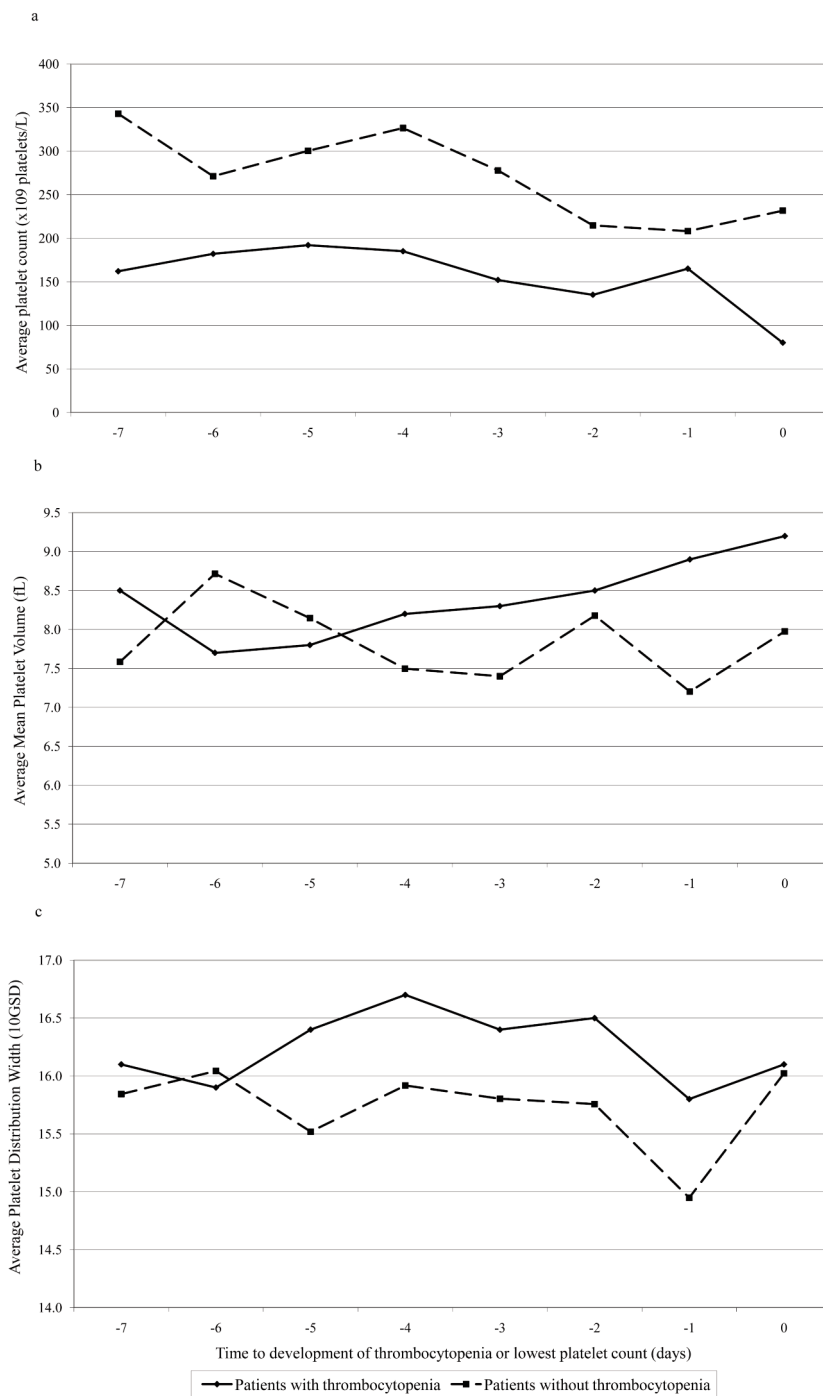
Based on a MPV higher than 9.5 fL 15 patients can be identified. In all of these patients MPV increased with at least 1.7 fL with a maximum increase of 6.7 fL. Patient 3 and 24 developed isolated thrombocytopenia and patients 2, 3, 19, 23, and 26 developed thrombocytopenia after re-exposure to rituximab which are proxy measures for immune-mediated destruction of platelets. In addition, patient 2 developed severe thrombocytopenia within 1 day after the administration of rituximab supporting an immune-mediated mechanism. For patient 26 an immune-mediated mechanism can be questioned since the MPV increased to 9.5 fL after administration of rituximab, which is the lower limit of the reference value used in the UMCU. In addition, the patient developed grade 1 thrombocytopenia and was concomitantly treated with cytostatics. The assumption of immune-mediated thrombocytopenia is only supported by the PDW in patients 18, 21, and 23, whereas the PDW did not change or decreased in all other patients.

The MPV of patient 1 is below 9.5 fL. However, the MPV and PDW increased after administration of rituximab and the patient developed isolated thrombocytopenia. Patients 4 to 7 might also indicate an immune-mediated mechanism based on the timing between administration of rituximab and the occurrence of thrombocytopenia. In addition, patients 6 and 7 developed isolated thrombocytopenia and patients 4 and 6 developed thrombocytopenia after re-exposure. However, in all cases the assumption of immune-mediated thrombocytopenia is not supported by the MPV and the PDW. Patient 5 had no other characteristics of immune-mediated thrombocytopenia and was therefore not classified as such. Patients 10 and 12 both had isolated thrombocytopenia and patient 10 developed thrombocytopenia after re-exposure to rituximab. In addition, the MPV in patient 10 increased with 1.7 fL. For patient 12, an immune-mediated mechanism seems less likely. Patients 11, 13, 17, 20, and 27 had no specific characteristics indicative for an immune-mediated mechanism.

Discussion

In this study we investigated several aspects of thrombocytopenia in patients treated with rituximab in the clinical setting, including the incidence, risk factors and early warning markers and we explored the underlying mechanism. We found a cumulative incidence of all grade thrombocytopenia of 30% and 20% for grade 3 and 4 thrombocytopenia within 30 days after the administration of rituximab. Use of rituximab in the oncology indication seemed to be a risk factor for the occurrence of thrombocytopenia. Patients who developed thrombocytopenia had a lower platelet count (217.5 vs. $324.4x10^9$ platelets/L) and a higher PDW (16.1 vs. 15.8) before administration of rituximab compared to the patients who did not develop thrombocytopenia. Patients who developed thrombocytopenia all had a platelet count below $200x10^9$ platelets/L in the week before the diagnosis of thrombocytopenia, whereas the platelet count remained above this value for all patients who did not develop thrombocytopenia. An immune-mechanism might have played a role in 19 of the 27 patients with thrombocytopenia.

Table 2: Characteristics of 27 patients developing thrombocytopenia[a].

Patients	Age (yrs)	Gender	Indication for use	Platelet nadir (x10^9)	Grade of thrombocytopenia nadir	MPV (fL) at nadir	ΔMPV[b] (fL)	PDW (10GSD) at nadir	ΔPDW[b] (10GSD)	Time between intake and platelet nadir	Isolated thrombocytopenia	Thrombocytopenia after first administration	Concomitant use of cytostatics	Time between start cytostatics and occurrence of platelet nadir	Concomitant use of medication associated with thrombocytopenia	Immune-mediated thrombocytopenia likely
1	1	M	Auto immune hemolytic anaemia	96	1	9.3	2.3	18.6	2.8	1 day	Yes	Yes	No	-	Yes	Yes
2	61	F	NHL	34	3	13.0	6.3	14.7	-0.8	1 day	No	No	Yes	10 days	Yes	Yes
3	42	M	Polyneuropathy	72	2	10.4	2.1	16.5	-0.2	2 days	Yes	No	No	-	Yes	Yes
4	43	M	NHL	46	3	9.3	-0.8	15.6	-0.4	2 days	No	No	No	-	Yes	Yes
5	23	M	NHL	48	3	8.9	0.8	15.0	-1.0	4 days	No	Yes	Yes	10 days	Yes	No
6	50	M	Poly arthritis	83	1	8.9	0.8	14.7	-2.5	4 days	Yes	No	No	-	Yes	Yes
7	58	F	NHL	90	1	8.8	-0.7	15.9	-1.1	4 days	Yes	Yes	No	-	No	Yes
8	45	M	Kaposi sarcoma	15	4	10.0	1.9	10.0	-7.0	5 days	No	Yes	No	-	Yes	Yes
9	76	F	NHL	94	1	11.3	1.7	15.1	-2.5	5 days	No	Yes	Yes	5 days	Yes	Yes
10	48	F	NHL	78	1	8.7	1.7	15.6	0.0	6 days	Yes	No	Yes	5 days	Yes	Yes
11	22	M	NHL	69	2	8.9	0.6	16.8	0.9	7 days	No	Yes	No	-	Yes	No
12	42	M	NHL	53	2	8.0	0.6	15.4	-0.5	8 days	Yes	Yes	Yes	13 days	No	No
13	35	F	NHL	46	3	8.0	0.6	14.5	-1.9	9 days	No	Yes	No	-	Yes	No
14	54	M	NHL	31	3	11.2	4.3	15.5	-1.0	9 days	No	Yes	No	-	Yes	Yes
15	65	F	NHL	25	4	10.2	3.2	16.1	0.1	9 days	No	Yes	Yes	9 days	Yes	Yes
16	84	M	NHL	10	4	15.8	6.3	10.3	-6.7	9 days	No	Yes	Yes	9 days	Yes	Yes
17	37	M	NHL	30	3	9.4	2.6	15.0	-1.3	10 days	No	Yes	Yes	12 days	Yes	No

18	55	F	NHL	24	4	**10.4**	**2.6**	**18.5**	**3.2**	10 days	No	Yes	**No**	-	Yes	Yes
19	66	F	NHL	28	3	**11.0**	**3.0**	**16.3**	**-1.5**	10 days	No	No	**No**	-	Yes	Yes
20	40	M	NHL	49	3	7.6	0.2	16.7	0.0	11 days	No	Yes	No	-	Yes	No
21	51	M	NHL	39	3	**10.2**	**3.9**	**16.9**	**1.4**	11 days	No	Yes	**No**	-	No	Yes
22	56	M	NHL	7	4	**13.1**	**6.6**	10.4	-5.5	11 days	No	Yes	**No**	-	Yes	Yes
23	61	M	NHL	43	3	**12.3**	**5.0**	**16.4**	**1.1**	12 days	No	No	**No**	-	Yes	Yes
24	11	M	NHL	15	3	**16.1**	**6.7**	10.3	-5.1	13 days	**Yes**	Yes	Yes	19 days	Yes	Yes
25	1	F	Dermatomyositis	11	4	**14.3**	**5.2**	10.3	-6.6	15 days	No	Yes	Yes	9 days	Yes	Yes
26	64	F	NHL	92	1	**9.5**	**2.0**	15.5	-1.7	17 days	No	**No**	Yes	17 days	Yes	No
27	46	F	NHL	46	3	**9.2**	**1.2**	14.5	-1.7	27 days	No	Yes	Yes	9 days	Yes	No

a) The characteristics presented in bold are characteristics of immune-mediated thrombocytopenia

b) Δ Represents the difference in MPV and PDW measured before administration of rituximab and at the event measurement

F = Female; GSD = Geometric standard deviation; M = Male; MPV = Mean platelet volume; NHL = Non-Hodgkin Lymphoma; PDW = Platelet distribution width.

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This study provides important information on the incidence of thrombocytopenia using rituximab in a clinical setting. The estimated grade 3 and 4 cumulative incidence calculated within the present study is much higher as compared to the incidence observed within the clinical trials in which rituximab was administered in combination with chemotherapy. In these studies 15% of the patients developed thrombocytopenia. In the clinical trials in which rituximab was administered as monotherapy 1.7% of the patients developed thrombocytopenia.[5,6] Exclusion of the patients concomitantly treated with cytostatics in our study results in an estimated cumulative incidence of grade 3/4 thrombocytopenia of 19.6% which is higher compared to the 1.7% found in the clinical trials. The calculated cumulative incidence is based on the 90 patients included in the study. Patients with potential signs of thrombocytopenia, e.g. petechia, bruising, epistaxis, and/ or bleeding,[1] might have been measured more frequently compared to patients without symptoms of thrombocytopenia. Exclusion of the patients without a complete blood count might, therefore, have led to selection bias, which will have resulted in an overestimation of the calculated cumulative incidence. An estimate of the cumulative incidence with all 235 patients treated with rituximab gives 11.5% for all grade and 7.7% for grade 3 and 4 thrombocytopenia, which remains higher as compared to the incidences found in the clinical trials. Healthcare professionals should consider thrombocytopenia as a relevant complication of rituximab treatment.

Patients treated with drugs in the clinical setting are known to be different compared to the population included in the clinical trials.[7] Data obtained during use of drugs in the 'real-world' clinical setting can, therefore, add important information regarding the safety of drugs. This is underlined by the findings of the present study. Data sources like UPOD contain important information collected during the routine use of drugs in clinical practice and will be important to study the safety of drugs after entering the market.[16] In addition, due to the structure of UPOD data were not only available on laboratory markers but also on the use of concomitant medication and patient and disease characteristics, which increased the value of this study.

Identification of risk factors for the development of an ADR is important to identify patients at an increased risk before treatment is initiated. We identified patients treated with rituximab in the oncology indication to have an almost 5 times higher risk for the development of thrombocytopenia as compared to patients treated with rituximab for auto-immune diseases. Although, this finding was not statistically significant in the multivariate model, treatment with rituximab within the oncology indication is likely to be a risk factor for rituximab-induced thrombocytopenia. Interestingly, concomitant treatment with cytostatic agents was not identified as a risk factor in the model. Previous case reports have identified bone marrow involvement as a potential risk factor, which might (partly) explain the increased risk seen in oncology patients in the present study.[8] However, no data was available on bone marrow involvement neither on the other potential risk factor: splenomegaly.[9] Concomitant use of cytostatics and use of any other drug known to be related to thrombocytopenia was not identified as a risk factor in the univariate model. Concomitant use of fluconazole and ciprofloxacin were identified as potential risk factors in the univariate model. Fluoroquinolone-induced thrombocytopenia has been described in literature.[21,22]

Patients developing thrombocytopenia had a higher PDW and a lower platelet count before administration of rituximab. With regard to the early warning markers, patients developing thrombocytopenia had an average platelet count below $200x10^9$ platelets/L in the week preceding the first measurement of thrombocytopenia, whereas the platelet count remained above $200x10^9$ platelets/L in the week before the lowest platelet count was reached for the controls.

Knowledge on risk factors and potential early warning markers may trigger more intensive monitoring in patients with known risk factors resulting in the identification of a drop in platelet counts at an earlier stage. This seems relevant due to the high incidence of thrombocytopenia during use with rituximab found in the present study. The management of DIT with for example corticosteroids, immunoglobulines, and/ or platelet transfusion is still controversial.[2,3] Within the present study the concomitant use of corticosteroids within the 14 days before the platelet count dropped below $100x10^9$ platelets/L was not identified as a protective factor against the development of rituximab-induced thrombocytopenia. A recent case report described a patient who developed acute severe thrombocytopenia after every administration of rituximab which improved spontaneously over 2-3 days on every occasion.[23] Further research into the management of DIT is needed.

The mechanism of rituximab-induced thrombocytopenia was explored using different characteristics including the MPV and PDW. MPV and PDW have been shown to be of discriminative value in patients with thrombocytopenia resulting from a decreased platelet production, e.g. aplastic anaemia, and thrombocytopenia resulting from an immune mechanism, e.g. idiopathic thrombocytopenic purpura.[11-13] Studies evaluating these parameters did not reach the same conclusion regarding the parameter which is the most reliable to discriminate between immune-mediated thrombocytopenia and thrombocytopenia as a result of bone marrow suppression.[12,13] A study by Bowles et al only studied the MPV and concluded that the MPV can strongly guide the clinician to the presence or absence of bone marrow disease in thrombocytopenic patients.[19] Within the present study it was therefore decided to mainly focus on the MPV. In addition to the MPV, other proxy measures suggestive for immune-mediated thrombocytopenia were used. The characteristics used in the present study can only be considered suggestive for an immune-mediated mechanism. Based on the patients with thrombocytopenia it is suggested that an immune-mediated mechanism plays a role in rituximab-induced thrombocytopenia.

This study has several limitations which need to be addressed. Firstly, thrombocytopenia after re-exposure to a drug is supportive of an immune-mediated mechanism and was used to explore the mechanism of rituximab-induced thrombocytopenia. As already described in the methods section, no electronic data is available on drug exposure before January 2004 and the method used to identify previously treated patients might not have been complete. In addition, patients might have been treated with rituximab as part of a double-blind controlled trial lacking knowledge on actual exposure to rituximab. Although this limitation is acknowledged, we feel that this does not affect our conclusion since we used a combination of proxy markers for immune-mediated thrombocytopenia, e.g. MPV, timing between administrations of rituximab and isolated vs. non-isolated thrombocytopenia. Secondly, since UPOD is a hospital based registry, data on drugs used in the

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home setting might not have been complete.

In conclusion, we found that one out of three patients treated with rituximab in clinical practice developed thrombocytopenia and one out of five of the patients developed grade 3 and 4 thrombocytopenia, which is higher compared to the incidences seen during the clinical trials. Healthcare professional should consider thrombocytopenia as a relevant ADR during treatment with rituximab. More frequent monitoring of the platelet count is especially advised in patients treated with rituximab in the oncology indications. An initial platelet count below $200x10^9$ platelets/L might be indicative for the development of thrombocytopenia and these patients may be monitored more intensively. More intensive monitoring may result in the identification of a drop in platelet counts at an earlier stage.

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Appendix 1: List of drugs associated with thrombocytopenia and used by the patients included in the study.
[Available from URL: http://www.ouhsc.edu/platelets. Accessed 20 September 2010].

Allopurinol
Amiodaron
Atorvastatine
Carbamazepine
Ceftazidime
Ceftriaxone
Ciprofloxacine
Cyclosporine
Diazepam
Diclofenac
Digoxin
Enalapril
Fluconazole
Haloperidol
Heparin/ Low-molecular weight heparin
Hydrochlorothiazide
Lopinavir
Methotrexate
Naproxen
Ondansetron
Paracetamol
Phenytoin
Ranitidine
Rifampin
Simvastatine
Sulfamethoxazole/ trimethoprim
Tamoxifen
Vancomycin

Appendix 2: List of ICD-9-CM codes. Based on this list, patients likely to have been treated with rituximab before 2004 were selected and the letters of the specialist to the GP were checked if mention was made of previous exposure.

ICD-9 code	Corresponding indication
201.90	Hodgkin's disease, unspecified
202.80	Other lymphomas
202.83	Other lymphomas; intra-abdominal lymph nodes
204.10	Chronic lymphoid leukemia
357.40	Polyneuropathy in other diseases classified elsewhere
581.30	Nephrotic syndrome with lesion of minimal change glomerulonephritis
581.90	Nephrotic syndrome with unspecified pathological lesion in kidney
590.80	Pyelonephritis, unspecified
710.30	Dermatomyositis
714.00	Rheumatoid arthritis and other inflammatory polyarthropathies
714.07	Rheumatoid arthritis and other inflammatory polyarthropathies
715.21	Osteoarthritis, localized, secondary; localized 2nd osteoarthrosis - shoulder
715.24	Osteoarthritis, localized, secondary; hand, Carpus, Metacarpus, Phalanges
715.27	Osteoarthritis, localized, secondary; ankle and foot, Ankle joint, Digits [toes], Metatarsus, Phalanges, foot, Tarsus, Other joints in foot
715.36	Osteoarthritis, localized, not specified; lower leg, Knee joint, Patella, Tibia
721.00	Spondylosis and allied disorders

Chapter 5

General discussion

Introduction

In this thesis, the risk management of biologicals was studied from a regulatory and a clinical perspective. Biologicals have found their way in clinical practice as important innovative treatment options for a variety of chronic and serious conditions.[1-3] However, as is the case for every drug, biologicals are associated with (serious) adverse drug reactions (ADRs), some of which have been identified in the post-marketing setting. Biologicals have different characteristics as compared to the traditional small molecule drugs that create additional challenges for the risk management of these agents.

As we have seen in earlier chapters of this thesis, post-marketing identified ADRs of biologicals are often related to the pharmacological activity or to an immunogenic reaction related to the protein nature of these agents. This can be illustrated by some landmark examples related to the safety of biologicals such as the clearly increased incidence of pure red cell aplasia (PRCA) after a change in the manufacturing process of epoetin alfa[4,5] and the identification of severe infections with some of the biologicals with an immuno-suppressive effect.[6] The increased incidence of PRCA closely relates to one of the main characteristics of biologicals, which is their high potential for immunogenicity. PRCA occurred after the formation of antibodies, which were not only directed against the administered epoetin alfa but also against the endogenous available protein.[4,5] A typical example of an ADR related to the pharmacology can be illustrated by the occurrence of tuberculosis after use of the tumour necrosis factor alfa (TNF-α) inhibitors.[7,8] This issue was first described for infliximab after an analysis of the US FDA spontaneous reporting system MedWatch.[8] TNF-α was identified to play an important role in the human immune defence against *Mycobacterium tuberculosis.*[7] Current clinical guidelines include the recommendation that patients should be tested for latent tuberculosis before treatment with TNF-α is initiated and, if latent tuberculosis is shown, this should be eradicated first.[9,10] A second example of a serious infection occurring after the use of biologicals are three confirmed case reports of progressive multifocal leukoencephalopathy (PML) with the use of efalizumab, two of which were detected via spontaneous reporting. In addition to what was already known about the risks and benefits of efalizumab, these cases of PML made the European regulators conclude that the benefits no longer outweighed the risk, which led to withdrawal of the drug from the market.[11] These cases illustrate that monitoring the safety of drugs should be a continuous process, which starts at the moment of the preclinical studies and continues until the end of the marketing life of the drug.

Monitoring the safety of drugs during the marketing life has changed from a reactive towards a more proactive approach as described in the guideline on pharmacovigilance planning.[12] This guideline by the International Conference on Harmonisation (ICH) has been implemented in the EU, resulting in the obligatory submission of an EU-Risk Management Plan (EU-RMP) at the moment of a marketing application, as was described in the introduction of this thesis.[13,14] The need for proactive pharmacoepidemiological studies to further characterise the safety profile of biologicals is underlined by the findings in *chapter 2.1*. This chapter showed that safety concerns for biologicals were less frequently classified as important identified risk and more frequently as important missing information compared to the traditional small molecule drugs. Although the role of the EU-RMP in pro-active risk management is acknowledged, the study presented in *chapter 2.1* also

showed that submissions of more complete study protocols at the moment of regulatory approval are warranted. In addition, it was concluded that differences exist between biologicals and small molecules, e.g. in the type of safety issue to be studied and the data source proposed for post-authorisation pharmacoepidemiological studies. Based on the results found in *chapter 2.1* several challenges in the conduct of pharmacoepidemiological studies for biologicals might be expected, e.g. the type of safety concerns and the data source to be used. Challenges in the assessment of the hazard function of biologicals and the susceptibility for ADRs of patients treated with these agents are also anticipated. These challenges will be discussed in depth within this chapter and, where appropriate, the studies in this thesis are placed in a broader context. In addition, the use of risk factors and early warning markers in clinical practice are discussed.

Five themes will be discussed:

• Exposure and outcome assessment of biologicals
• Data sources for the conduct of pharmacoepidemiological studies of biologicals
• Assessment of the hazard function of biologicals
• Susceptibility for ADRs of patients treated with biologicals
• Recommendations for regulatory and clinical practice

Exposure and outcome assessment of biologicals

Epidemiological studies aim to evaluate the occurrence of certain health outcomes of interest (outcome) as a function of certain determinants (exposure).[15] Definition of the determinant and information on the outcome is very important in pharmacoepidemiological studies and specific aspects related to biologicals will be discussed in this chapter.

The exposure – classification of biologicals

In pharmacoepidemiological studies a specific drug or a group of drugs can be studied. Both approaches were used in this thesis. In **chapter 4** a cohort of rituximab users was established and followed to identify risk factors and early markers for the early identification of patients at risk for a certain ADR. The studies presented in **chapter 4** will be discussed in a later section of this discussion.

Within *chapters 2.2* and *3.1* we studied biologicals as a group according to the commonality in their mechanism. The mechanistic classification is based on a combination of the main target of the biological and/ or the structure of the biological. This resulted in eight classes: antibodies, cytokines, enzymes, hormones, growth factors, interferons, receptors, and others/ various. Within these studies we used hormones as the reference since this class includes the biologicals for which most experience is gained. *Chapter 3.1* showed that the safety profile differed among the mechanistic classes. ADRs related to neoplasms were, for example, more frequently reported for antibodies and receptors. From a pharmacovigilance perspective it is relevant to be able to predict certain ADRs based on the characteristics of the biological. A classification based on the pharmacology is relevant. A new classification system has recently been described by Leader et al. They propose four main categories: Group I are protein therapeutics with enzymatic or regulatory activity, Group II are protein therapeutics with special targeting activity, Group III are

protein vaccines, and Group IV are protein diagnostics. These groups are further classified in subgroups (Box 1). This classification is a first step[16] towards mode of action driven safety assessment and has to prove its value for pharmacovigilance. A more detailed classification of biologicals according to their specific mode of action can further add to the prediction of ADRs based on the pharmacology. Pre-registration clinical trials and post-marketing studies can then specifically be targeted towards the identification of these expected type A ADRs, e.g. tuberculosis with the use of the TNF-α inhibitors.[7-10] Mode of action driven safety assessment is, however, complicated by the often complex mode of action of many biologicals and the lack of knowledge on the role of specific cells and cytokines in the human immune defence.

Box 1: Functional classification of protein therapeutics.[16]

Group I: protein therapeutics with enzymatic or regulatory activity

- Ia: Replacing a protein that is deficient or abnormal.
- Ib: Augmenting an existing pathway.
- Ic: Providing a novel function or activity.

Endocrine and metabolic disorders with defined molecular aetiologies dominate Group Ia. As more diseases are linked to deficiencies of specific proteins, this class will continue to grow. Group Ib is dominated by therapies that augment haematological and endocrine pathways and immune responses. The many interferon and growth factor therapies in Group Ib effectively treat disease even when their precise pharmacological mechanism of action is unknown. Group Ic demonstrates the rational use of naturally occurring proteins to modify the pathophysiology of human diseases.

Group II : protein therapeutics with special targeting activity

- IIa: Interfering with a molecule or organism.
- IIb: Delivering other compounds or proteins.

Group IIa therapeutics use their special targeting activity to interfere with molecules or organisms by binding specifically to them and blocking their function, targeting them for destruction, or stimulating a signalling pathway. This group has grown as monoclonal antibody technology has matured and will probably expand further as signalling pathways and aetiologies of disease are more clearly identified. Group IIb therapeutics deliver other compounds or proteins to a specific site.

Group III : protein vaccines

• IIIa: Protecting against a deleterious foreign agent.
• IIIb: Treating an autoimmune disease.
• IIIc: Treating cancer.

Although this is currently a small class of therapies, there is great potential for the production of recombinant vaccines that provide broad protection against infectious agents. Similarly, individualized vaccines against cancers are likely to be in great demand.

Group IV : protein diagnostics

Group IV protein diagnostics are a class that powerfully affect clinical decision-making. These diagnostics use technology and therapeutics developed in other classes to answer clinical questions.

In addition to the classification of biologicals as a group there is increasing interest in differences between biologicals with the same active substance. This is mainly based on the registration of "biosimilars", which are also called "biogenerics" or "follow-on biologics".[17] Due to the complexity of the production process of a biological, it is impossible to produce an exact copy of the reference product.[17] This does not only imply that innovator products are not the same as biosimilars but also a change in the production process or a change in storage conditions within one biological can affect the ADR profile. This

is mostly reflected in a difference in immunogenicity of the product. The cases of PRCA with the use of epoetin-α were related to a change in the production process which un-expectedly altered the immunogenicity of the product.[4,5] This case can, therefore, be con-sidered a type B reaction for biologicals The occurrence of PRCA with epoetin alpha had impact on the approval of the so-called biosimilar epoetins. Biosimilar epoetins should particularly focus on PRCA in their EU-RMP.[18] If a patient develops a certain immuno-logical reaction after administration of a biological it is very important to identify the re-sponsible product and the responsible batch of a specific product. This is complicated by the current naming system of drugs by the World Health Organisation (WHO) since biosimilars receive the same international non-proprietary name (INN) as the reference product.[17,19] To be able to trace the product associated with the ADR, biosimilars should receive a name by which they can be clearly identified and distinguished from the refer-ence product and other biosimilars.[20] In addition, dedicated forms should be designed to collect additional information during the reporting of ADRs, e.g. collection of batch num-bers. Future research should focus on the identification of the product administered in different data sources, for example in a spontaneous data source and in registries. New developments in this area (e.g. use of barcodes) may offer solutions in future.[21]

The outcome – frequency and nature of ADRs

We have studied the probability for a first safety-related regulatory action, defined as a communication to healthcare professionals and/ or a black box warning, for biologicals and orphan drugs after marketing. In *chapter 2.2* we found that biologicals have a prob-ability of receiving a first safety-related regulatory action of 14% the first three years after approval. This had increased to 29% after ten years. In *chapter 2.3* we found an overall probability for all orphan drug of 3.5% after three years of follow-up and 20.3% after 8 years of follow-up. A study by Lasser et al identified that new chemical entities had a probability of a black box warning of 10% after 10 years of marketing.[22] This might suggest that biologicals are not as safe as the small molecule drugs, which were mostly included in the study by Lasser et al. However, several differences between both studies have been identified. Lasser et al included multiple black box warnings issued for the same drug and there is a delay in the occurrence of a black box warning in the *Physicians' Desk Reference*. In addition, there has been an increasing awareness of patient safety and accessibility of safety data over time. This is reflected in the number of communications to healthcare professionals, which increased by 2.1 letters per year over the past decade.[23] This precludes a direct comparison between both studies. Within the study presented in *chapter 2.3* we concluded that biological orphan drugs do not have a higher risk for a first safety-related regulatory action as compared to the small molecule orphan drugs. This might lead to the conclusion that safety-related regulatory action are issued with a comparable frequency for biologicals and the traditional small molecule drugs. However, further research is needed to compare the frequency of post-marketing identified ADRs between biologicals and small molecules. A study by Mol et al. showed that biologicals were associated with more rapidly issued communications to healthcare professionals as compared to the small molecule drugs.[23] As shown in *chapter 2.2* biologicals that were the first to be approved in a new ATC class were at a higher risk for a first safety-related

regulatory action. Pharmacovigilance should, therefore, especially be stringent for the first biological approved in a new ATC class. Information obtained with the first biological to be approved in a new ATC class should be applied to other biologicals approved in the same ATC class. This so-called regulatory learning was shown by Arnardottir et al for HIV drugs. Arnardottir et al showed that ADRs identified in the post-marketing setting were included in the registration process of HIV drugs in the same ATC-4 class.[24]

Biologicals have a high potential for immunogenicity and the ADRs can often be attributed to the pharmacological activity.[3,6] This is reflected in the studies presented in *chapters 2.1, 2.2* and *3.1.* In *chapter 2.2* we found that most safety-related regulatory actions for biologicals are related to infections, immunological reactions and neoplasms. Infections and neoplasms can often be attributed to the immunosuppressive effect of many biologicals whereas immunological reactions are related to the protein nature of these agents. This is different from the ADRs frequently identified for the traditional small molecule drugs, which are often related to the liver, the blood system, the cardiac system and the nervous system.[22,25,26] This difference in the safety profile was confirmed in the study described in *chapter 3.1.* In that study we used the WHO-ADR database VigiBase, from which spontaneously reported suspected ADRs for biologicals and small molecules were selected and classified at the System Organ Class (SOC) level of the Medical Dictionary for Regulatory Authorities (MedDRA). It was confirmed that suspected ADRs for biologicals were more frequently reported in the classes related to infections and neoplasms and in the classes which contain ADRs related to immunological events. A potential challenge in the identification of ADRs related to immunological events is the wide variety of ADRs which are indicative of an immunological reaction. Immunogenicity is most frequently expressed as a lack of efficacy in case neutralising antibodies bind to the biological and neutralise its biological activity or influence its biological. However, an altered immunogenic profile can result in (serious) ADRs, as described earlier in this chapter by the increased incidence of PRCA after a change in formulation of epoetin alpha.[5,17,27] Within the study presented in *chapter 3.1* we identified that 17% of the reported ADRs, which potentially indicate an immunological reaction, are definitely an immunological reaction. This involved ADR terms as hypersensitivity, antibody test positive etc, whereas 83% of the ADR terms, for example fever and hypotension, might indicate an immunological reaction but this can not be established with absolute certainty.

The difference in the safety profile of biologicals as compared to the traditional small molecule drugs was also identified before approval of the drug. In *chapter 2.1* we performed an analysis of EU-RMPs at the moment of regulatory approval and found that safety concerns to be studied in pharmacoepidemiological studies of biologicals frequently relate to infections and immunological reactions. Immunological reactions should always be classified as at least an important potential risk in the EU-RMP for biologicals. As shown in *chapter 2.1* ADRs related to the development of neoplasms were more frequently studied in the post-marketing setting for small molecule drugs. ADRs related to neoplasms are, however, also important potential safety concerns for biologicals and should be carefully considered in the EU-RMP. In *chapter 2.3* we studied the safety-related regulatory actions issued for orphan drugs and found that two of the actions issued for biologicals involved ADRs related to immunological events. In contrast to the results

presented in *chapter 2.2* and *3.1*, two actions were issued related to neoplasms for small molecule drugs. This might be due to different classes of biologicals involved in the treatment of orphan diseases. There are, for example, more orphan biologicals with enzymatic activity and less with an immunosuppressive effect.[28]

The results presented here have shown that ADRs for biologicals identified in the post-marketing setting are frequently related to an immunological response and to their pharmacological activity. In addition, applicability of the traditional classification system of ADRs in type A and B can be questioned for biologicals. The traditional type B ADRs often include immunological reactions and can be expected for biologicals based on their protein nature. These considerations made Pichler decide to propose a new classification system of ADRs for the biological agents belonging to the cytokines, antibodies and fusion proteins (Figure 1).[29] In addition, it is important to identify clusters of related ADRs for pharmacovigilance purposes. The study presented in *chapter 3.2* aimed to qualify and quantify how spontaneously reported ADRs fit the classification system as proposed by Pichler. It was concluded that certain clusters of related ADRs were identified. These clusters of ADRs can be used for signal detection of biologicals.

Figure 1: Proposed classification system for adverse drug reactions of biologicals[29.]
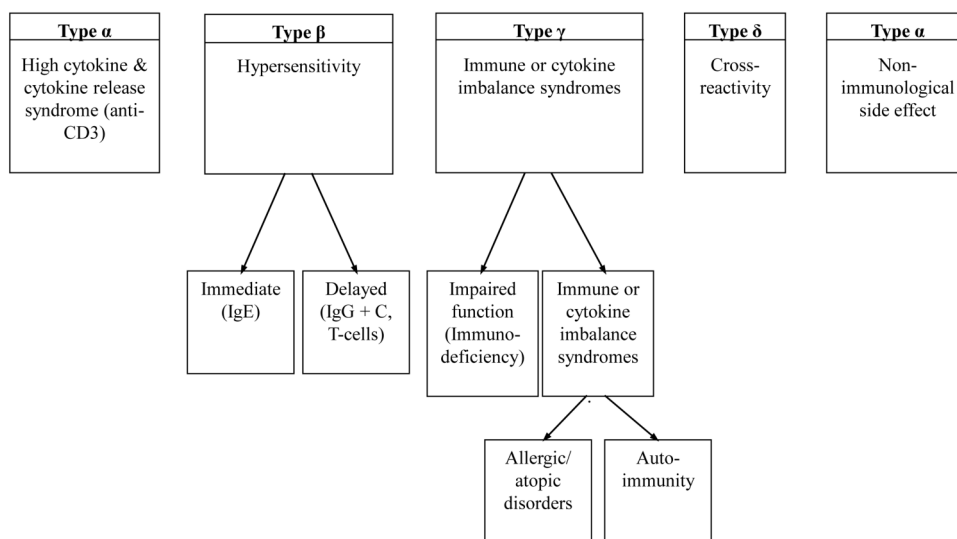
Data sources for the conduct of pharmacoepidemiological studies of biologicals

For the conduct of pharmacoepidemiological studies the database to be used should contain the information needed to address the research question. This should be carefully addressed during the design phase of the study. Several data sources can be used for pharmacoepidemiological studies including spontaneously reported ADRs, large population based databases and registries. Within this thesis the WHO-ADR database VigiBase and the Utrecht Patient Oriented Database (UPOD) were used. In addition, to these databases use of registries and large population based databases are discussed.

Databases in which spontaneously reported ADRs are collected are especially important with regard to the identification of (very) rare ADRs, which will only be identified after a large number of patients have been exposed to the drug.[30,31] Data from these databases can be used for the identification of potential safety signals, either by the traditional case-by-case approach or for quantitative signal detection.[32] The traditional case-by-case approach was used for the potential relation between infliximab and the occurrence of tuberculosis, as described previously.[8] Quantitative signal detection, on the other hand, is based on the use of statistical techniques to select drug-adverse event pairs which stand out against the background of the database. The total number of ADRs reported for a specific drug are used as an estimation of the total number of patients exposed in the denominator of the formula. Several measures of disproportionality, such as the reporting odds ratio (ROR) and the proportional reporting ratio (PRR) are being used.[33,34]

Within *chapter 3.1* we used the PRR to compare the ADRs reported for biologicals with the ADRs reported for small molecule drugs and found that certain ADRs, e.g. ADRs related to infections and neoplasms, were more frequently reported for biologicals. This information should be used during pharmacovigilance planning of related products. The signals obtained with both methods of signal detection can be used for signal generation and hypothesis strengthening.[33] Petitpain et al, for example, described 85 spontaneously reported arterial and venous thromboembolic events during anti-TNF therapy and concluded that venous thrombosis could be related to the use of TNF-α antagonists. However, further confirmation by controlled studies is needed, as concluded by the authors.[35] Although spontaneous reporting is important for signal detection and hypothesis generation, several limitations need to be addressed. Underreporting is a major problem and is expected to be in excess of 90%.[30] In contrast to underreporting, selective overreporting might also occur as a result of, for example, a regulatory action or media attention.[36] This was shown by De Bruin et al, who illustrated how media attention to anti-histamine induced arrhythmias biased the association between drug exposure and outcome.[37] Another limitation is that a detailed description of the case and knowledge of (confounding) risk factors is often lacking.[38] In the study presented in *chapter 3.1*, it was shown, for example, that information on sex was missing in 3.7% of the reports for biologicals and 6.6% of the reports for small molecule drugs. Although the limitations of spontaneous databases are acknowledged, information gathered this way has proven to be an important and continuous source of information on suspected ADRs and other drug-related problems.[39] We, therefore, used spontaneously reported ADRs in the study presented in *chapter 3.2,* in which we evaluated a new classification system of ADRs of biologicals. Spontaneously

reported ADRs were used as a first indication of how these ADRs fit the proposed classification system.

Signals obtained with spontaneous reporting can be further studied in disease and drug-related registries, which have shown to be important data sources for the conduct of pharmacoepidemiological studies of biologicals.[40-44] Large population based databases often include data from community pharmacies and general practitioners. Biologicals are, however, mostly used in the hospital setting and large population based databases are expected to contain limited information on biologicals. This is reflected in the pharmacoepidemiological studies proposed by pharmaceutical companies in their EU-RMP. We showed in *chapter 2.1* that post-marketing safety studies for biologicals are more frequently proposed in registries as compared to post-authorisation safety studies for the traditional small molecule drugs, which often use large population based databases for the conduct of their pharmacoepidemiological studies. To date, most experience has been gained with the registries in which biologicals used to treat rheumatoid arthritis (RA) are captured.

Within Europe there are eight RA registries among different countries and there are several registries in the USA capturing patients with rheumatoid arthritis treated with biologicals.[42,43] These registries have added important information on the safety of these agents. However, conflicting results between different registries have been found, which has resulted in important methodological lessons learned from these registries.[42,45,46] For example, two registries studied the risk of serious infections with TNF-α inhibitors and found no increased risk in one registry up to a twofold increased risk in another registry.[41,47-49] This has led to a discussion on the points to consider when establishing, analysing and reporting safety data of biologics registers in rheumatology. Although these points should generally be considered in the conduct of pharmacoepidemiological studies, it provides a clear overview based on the experience gained with the RA registries and these points are also applicable to registries in other disease and drug areas. Points to consider include, for example, 1) the population to be targeted; capture incident or prevalent users, define the exposed population and the intended comparator, 2) data items to be collected; clearly define the data to be collected both at the exposure and the outcome, and 3) follow-up methods; methods of follow-up should be similar between the exposed and the reference group, consider strategies to minimise loss to follow-up.[45] Pharmaceutical companies, regulators and academia should carefully consider these points when establishing a registry and assessing the study protocols submitted as part of the EU-RMP. A second example of a disease based registry is the Swedish multiple sclerosis (MS)-registry. In this registry, patients treated with the biological agent natalizumab are captured and followed-up. An analysis of 1,115 patients treated with natalizumab until January 2010 demonstrated good tolerability and efficacy in patients with severe MS. However, among the treated patients, three cases of PML have been identified which remains a concern with the use of natalizumab.[44] A drug based registry captures natalizumab users in Italy with the aim to obtain information on the utilisation and safety. After two years, 1,818 patients were registered in the database and the most frequently reported ADRs included hypersensitivity reactions and infections.[50]

In addition to registries case-control surveillance studies can add important information on the safety of biologicals. Case-control surveillance studies aim to actively include

patients with a certain ADR of interest and information is obtained on drug exposure. Controls are, for example, selected from the same (hospital) population and can be matched on certain characteristics. This method has especially shown its value for very rare ADRs, where it is extremely difficult to perform a case-control study in an established database including a sufficient number of patients.[51] This can be illustrated by the severe cutaneous adverse reactions (SCAR) study. The SCAR study aimed to evaluate and quantify the role of drugs and other factors in the development of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), to explore and quantify the etiologic role of various potentially predisposing factors, and to develop standardised clinical definitions of these diseases suitable for use in epidemiologic studies.[51,52] This approach can also be applied to biologicals. PML, for example, is a very serious and very rare condition related to the use of certain biologicals.[44,53] A multinational study in which patients with PML are actively included can add important information regarding the disease and for the identification of patients at risk for this serious condition.

Within **chapter 4** we have used data from UPOD and showed the value of this database in the identification of risk factors and early markers for ADRs. UPOD contains complete and detailed data on patient characteristics, laboratory test results, medication orders, hospital discharge diagnoses and therapeutic procedures for all patients treated at the UMCU since January 2004 and during routine laboratory tests all haematological parameters are measured and collected in the database. UPOD therefore contains important information for the conduct of (pharmaco)epidemiological studies, including descriptive, prognostic, and etiologic studies.[54] The value of this database has been shown by the thesis of Maarten ten Berg, who studied drug-induced thrombocytopenia with the use of UPOD.[55] A limitation of this database is, however, that is covers only one hospital limiting the number of patients exposed.

Assessment of the hazard function of biologicals

During treatment with a specific drug the risk for a certain ADR might not be constant over time. Three biologics registries capturing patients with rheumatoid arthritis in Germany, Sweden, and the United Kingdom found comparable incidence rates for infections among patients treated with TNF-α inhibitors. However, differences in the relative risk (RR) estimates were found among the registries. The UK registry found an adjusted RR of 1.03 (95% CI 0.68-1.57) for serious infections during anti TNF-α therapy compared to the traditional disease-modifying anti-rheumatic drugs (DMARDs). The German registry found a non-significant increased risk of 2.3 (95% CI 0.9-5.4) for etanercept and of 2.1 (0.8-5.5) for infliximab as compared to treatment with traditional DMARDs.[41,47,49] A closer look at these results identified differences in the study population, the definition of 'serious infection', the calculation of 'time at risk' and the duration of follow-up. The duration of follow-up seemed to play an important role in the difference found.[40] This was confirmed by the results from the Swedish registry, in which a stratified analysis was conducted according to the duration of treatment. An increased risk was observed during the first year of treatment (RR 1.43; 95% CI 1.18-1.73) which decreased to 1.15 (95% CI 0.88-1.51) during the second year of treatment and to 0.82 (95% CI 0.62-1.08) for subjects who remained on their treatment for three years.[48] A second study in the British

registry has confirmed this finding. In this study an increased rate ratio of 4.6 (95% CI 1.8-11.9) was identified within the first 90 days after the start of TNF-α treatment, which decreased to 1.22 (95% CI 0.88-1.69) after almost a year of treatment as compared to patients receiving traditional DMARD therapy.[49] This might be partly due to the phenomenon described as "depletion of susceptibles". "Depletion of susceptibles", can be summarised as patients who remain on the drug are those who can tolerate it while those who are susceptible to ADRs will stop the drug and thereby select themselves out of the exposed cohort.[56] This phenomenon is important to consider in the interpretation of pharmacoepidemiological studies.

Different patterns of risk during treatment and after discontinuation of treatment have been postulated by Dixon et al (Figure 2).[49]

While on drug treatment there can be: 1) an increased risk at the start of therapy, 2) a constant risk with ongoing drug exposure, 3) an increased risk with cumulative drug exposure, or 4) a combination of the previous patterns. After treatment has discontinued there can be 1) an ongoing constant risk for a set lag window, 2) a linear decrease in risk back to baseline, 3) a non-linear decrease in risk back to baseline, and 4) different duration's of risk windows based on the half-life of each drug. The pattern of risk is likely to differ according to the ADR under study. An infusion-related reaction will mostly occur early within the treatment course, whereas a malignancy will take much longer to develop and may be related to cumulative drug exposure, e.g. a malignancy occurring within two weeks after initiation of treatment is likely not to be related to the administration of that specific agent.[49] It is, therefore, necessary to define an at-risk window, which is the period that a certain ADR should be attributed to the drug.[46,49] Van Staa et al showed that the period at which patients are considered at risk for peptic ulcer therapy during or after treatment with non-steriodal anti-inflammatory drugs influences the calculated risk estimate.[57] The definition of an at-risk window is complicated for biologicals due to their often prolonged pharmacodynamic effect.[1] Rituximab, for instance, depletes B-cells already after the first administration and it takes about 9-12 months after the last administration before the B-cells have reached their pre-treatment levels.[58,59] ADRs occurring within this time period might still be related to rituximab. It is difficult to establish which pattern of risk and the time at risk relates to the drug under study. This should be considered before a study is initiated and analysis at different moments in time while on and off treatment might add important information. However, there should be a sufficient number of patients to be able to conduct such analyses.

Within *chapter 4.1* we studied risk factors for the occurrence of invasive Aspergillosis in a cohort of patients treated with rituximab. Within this study we used a risk-window of 9 months related to the duration of B-cell depletion of rituximab. However, it was shown that all cases of Aspergillosis were identified within the first 6.5 months after administration. Within *chapter 4.2* we studied the occurrence of thrombocytopenia in relation to the administration of rituximab. Thrombocytopenia normally occurs within 30 days after administration of a drug and a prolonged risk-window seems to be less important. This is confirmed by the findings of our study since most of the cases occurred within 10 days after administration of rituximab. It should be noted that there are recent reports of delayed thrombocytopenia after administration of rituximab.[60]

Figure 2: Patterns of constancy of risk of infection while receiving treatment (on drug) and after discontinuation of treatment (off drug). a) Increased risk at start of therapy. b) Constant risk with ongoing drug exposure. c) Increasing risk with cumulative drug exposure. d) Combination of the risk patterns shown in a–c. e) Ongoing constant risk for set lag window after discontinuation of treatment (drug stop). f) Linear decrease in risk back to baseline. g) Nonlinear decrease in risk back to baseline. h) Differing durations of risk windows, based on the half-life of each drug[49].

Chapter 5
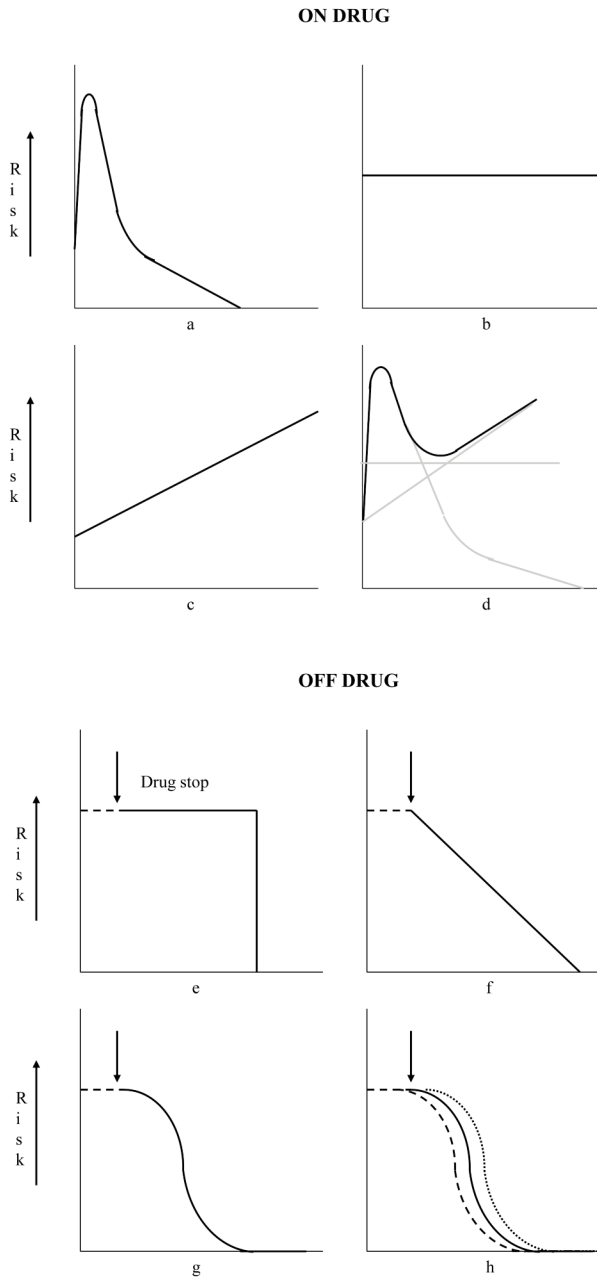
Susceptibility for ADRs of patients treated with biologicals

Therapy with biologicals is often indicated after treatment failure of one or more small molecule drugs. This is, for example, the case for the TNF-α inhibitors in the treatment of rheumatoid arthritis.[42] Within the EU these agents are approved in patients in whom the traditional DMARDs have not resulted in the expected benefit or in adult patients with severe, active and progressive disease not previously treated with DMARDs.[42] Rituximab is even indicated as third line treatment in these patients.[59] Pre-treatment with the traditional DMARDs might result in a higher susceptibility for ADRs during treatment with the biological agents. In addition, patients with rheumatoid arthritis are known to have an increased baseline risk for infections compared to the general population and measures reflecting the severity of rheumatoid arthritis were identified as strong predictors of infection risk in this population.[61,62] Since the biologicals are, in general, used in the more severely diseased patients, an increased risk for infections might also be (partly) related to a higher disease activity. This phenomenon is called channeling bias, which is the selective prescribing of drugs to patients with prognostic differences.[63] A second example of a serious ADR in which channeling bias might have played a role, is the withdrawal of efalizumab from the EU market as described in the introduction of this chapter.[11] Efalizumab was approved for the treatment of adult patients with moderate to severe chronic plaque psoriasis who had failed to respond to, or who had a contraindication, or were intolerant to other systemic therapies including ciclosporin, methotrexate and psoralen in combination with ultraviolet light A.[64] Channeling of efalizumab to the more severely diseased patients seemed likely in this case. At the moment that treatment with efalizumab was initiated the patient had already been treated with several other immunosuppressive agents. This probably made the patients more susceptible for the development of infections.

During the design phase of pharmacoepidemiological studies, the comparator group should be carefully selected and statistical methods may be helpful in the analysis phase to adjust for channeling bias. Propensity scores and instrumental variables might be applied to tackle the problem of channeling bias. Propensity scores reflect the probability of receiving a specific treatment based on prognostic baseline data. Patients treated with the drug of interest and the control group can then either be stratified based on their propensity score values or matching can be applied based on the propensity score of patients treated with the drug of interest and the control group.[65,66] A main limitation of propensity scores is that unobserved confounders can not be taken into account. Unobserved confounders can be taken into account with the use of instrumental variables. An instrumental variable is an observable factor associated with the actual treatment but not directly affecting outcome. Limitations of instrumental variables include the assumption that the instrumental variable only affects the outcome by being a predictor for the treatment assignment and not for the outcome, the treatment effect may not be generalisable to the population of patients whose treatment status was not determined by the instrumental variable, and when variation in the likelihood of receiving a particular therapy is small between groups of patients, differences in outcome may be very small and difficult to assess.[67,68]

Identification on risk factors and early markers is receiving increasing interest. This is valuable for the early identification of patients at high risk for the development of an ADR and to prevent and minimise the risk for the individual patients. The importance of risk factors can be illustrated by the recommendation that patients should be tested for latent tuberculosis before treatment with TNF-α is initiated and, if latent tuberculosis is shown, this should be eradicated first.[9,10]

This thesis contains two studies in which we aimed to identify risk factors and early markers for the early identification of patients at risk. Rituximab was used as a learning case in this context. In chapter 4.1 we aimed to identify risk factors for the identification of patients at risk for invasive Aspergillosis (IA). We found that patients treated with higher doses of rituximab and patients diagnosed with other fungal infections in the 30 days before the diagnosis of IA were at an increased risk for IA. These patients should especially be educated about activities to minimise the exposure to potentially invasive fungus and preventive treatment with anti-fungal therapy might be indicated in these patients. In chapter 4.2 we studied the occurrence of thrombocytopenia in relation to the use of rituximab. Thrombocytopenia is a known ADR of rituximab, which was already identified during the clinical trials. However, we found a cumulative incidence which was much higher as compared to the incidences found in clinical practice, which underlines the need for post-marketing safety evaluation of drugs. Case reports have described splenomegaly and bone marrow involvement as potential risk factors.[69,70] Our results showed that patients treated with rituximab in the oncology setting were at an increased risk for thrombocytopenia as well as patients with a relatively low platelet count at baseline and/ or after start of rituximab. Frequent monitoring of the thrombocyte count in these patients is advised.

Studies into the identification of risk factors and early markers should be encouraged. Use of risk factors and early markers to identify patients at risk for an ADR should receive more attention in pharmacovigilance and should already be taken into account during the clinical development of the drug. Information on risk factors and early markers should be clearly described in the Summary of Product Characteristics (SPC) to guide the physician and provide activities to minimise the risk for the individual patient.

Recommendations for regulatory and clinical practice

This thesis has studied several aspects related to the risk management of biologicals and leads to the following recommendations for regulatory and clinical practice:

- Biologicals differ in their safety profile as compared to the traditional small molecule drugs. ADRs identified for biologicals are frequently related to their pharmacological action, e.g. tuberculosis and TNF-α inhibitors, and to their high potential for immunogenicity, e.g. PRCA with epoetin. The pharmacology of the biological should be carefully studied during the development phase and should be used by regulators and industry to predict the ADR profile of the biological under assessment. Post-marketing pharmacoepidemiological studies should then specifically be targeted towards the identification of these ADRs.

Chapter 5

- Immunogenicity should always be considered as at least an important potential risk in the EU-RMP and healthcare professionals should be aware of the symptoms which might indicate an immunological reaction.

- Pharmacovigilance should especially be stringent for the first biological to be approved in a new ATC class. In addition, during assessment of a marketing application regulators should take into account the knowledge obtained with other biologicals with comparable characteristics.

- Activities should be implemented to increase the identifiability of the biological administered, e.g. specific names and collection of batch numbers at the moment of reporting of ADRs.

- Submission of more complete study protocols of post-marketing safety studies at the moment of regulatory approval is warranted for a proper regulatory assessment. In addition, follow-up on the study protocols and progress of the studies is needed.

- Case histories of individual patients, e.g. data sources containing spontaneously reported ADRs, will continue to be important for signal detection and hypothesis generation. Regulators should play an active role in signal detection with these type of databases. Healthcare professionals should continuously be educated to report ADRs to these databases.

- Registries have shown to be important data sources for the conduct of pharmacoepidemiological studies of biologicals for evaluation of signals. The Swedish ARTIS registry has shown the value of an active involvement of regulators in such a registry.

- The risk window and the pattern of risk should be carefully evaluated for ADRs related to biologicals and will be different between ADRs, e.g. an infusion reaction vs. a malignancy.

- Methodological challenges for pharmacoepidemiological studies of biologicals including a prolonged pharmacodynamic effect and channeling bias, e.g. efalizumab and PML, should specifically be considered during design, assessment and interpretation of study protocols and study results.

- Identification of risk factors and early warning markers for the identification of patients at risk for an ADR is relevant and should be implemented in regulatory and clinical risk management. This will minimise the risk for the individual patient, e.g. TNF-α and tuberculosis screening. Risk factors and early warning markers should be described in the Summary of Product Characteristics and regulators should more frequently ask for the identification of these factors in post-marketing safety studies.

Directions for future research
- Developments into mode of action driven safety assessment of biologicals should be continued.
- Efforts to design a new classification system of ADRs for biologicals should be encouraged.

- The role of the EU-RMP in the early identification of ADRs and the improvement of patient safety should be explored.
- The evolvement of study protocols submitted as part of the EU-RMP over time should be studied.
- Pharmacoepidemiological studies to address the methodological challenges, as outlined in this chapter, should be conducted.
- Risk factors and early warning markers to identify patients at risk for an ADR should receive more attention during the pre-registration clinical trials and pharmacoepidemiological studies. These risk factors and early markers should be implemented in clinical practice to increase the safe use of drugs. Activities to study the adherence of healthcare professionals to these recommendations should also be explored.

Conclusion

In conclusion, this thesis discusses the pharmacovigilance of biologicals with a strong focus on regulatory and clinical practice. Information on the characteristics of biologicals and their underlying ADR profile is important in regulatory and clinical risk management. Within regulatory risk management, the approach is changing from a reactive towards a more proactive approach and knowledge obtained with other biologicals should be used during the assessment of new biological agents. For clinical risk management, information on the safety profile of biologicals and use of risk factors and early warning markers can lead to a safer use of drugs in clinical practice and minimise the risk for the patient. Based on these considerations, we provided suggestions which might be helpful to improve the risk management of biologicals.

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Chapter 6

Summary & Samenvatting

Summary

Biologicals, also called biopharmaceuticals, are defined by the European Medicines Agency (EMA) as products which are produced by or extracted from a biological source and that need for its characterisation and the determination of its quality a combination of physico-chemical-biological testing together with the production process and its control. The introduction of recombinant DNA and hybridoma techniques in the 1980s enabled the large-scale production of biologicals. Nowadays, practice of medicine would be unthinkable without the availability of these compounds that have shaped important innovative treatment options for a variety of chronic and serious conditions, e.g. auto-immune diseases and cancer.

Biologicals have specific characteristics compared to the traditional small molecule drugs and therefore may carry specific risks. Differences include a very complex production and purification process for biologicals, a high potential for immunogenicity, a limited predictability of preclinical to clinical data due to species specific actions and immunogenic properties in animals, and toxicity which can often be attributed to the pharmacological activity of the biological.

Knowledge on the full safety profile of a drug is limited at the moment of marketing due to the limitations of randomised controlled trials, including, among others, a limited sample size and duration and a homogeneous population. In addition, regulators are more and more challenged by the need to balance rapid market access for new drugs with the wish for comprehensive safety data. The fact that approximately half of the biologicals are designated orphan drugs with limited pre-approval experience, further limits knowledge on the full safety profile at the moment of marketing for these drugs, in addition to the limitations as described above. Post marketing collected safety data offers a valuable and necessary complement to the clinical trials. However, specific challenges in the pharmacovigilance and pharmacoepidemiology of biologicals may exist based on their characteristics.

Regulatory actions and activities in the risk management of biologicals

Chapter 2 focuses on regulatory actions and activities in the risk management of biologicals. A relatively new regulatory activity is the obligatory submission of an European Union-Risk Management Plan (EU-RMP) as part of a marketing application for new chemical entities, including biologicals. In *chapter 2.1* we examined the types of proposed pharmacovigilance activities in a sample of EU-RMPs and described and evaluated the methodology of post-authorisation safety studies (PASS). Problems with PASS were identified and remedies were proposed. Within this study characteristics of biologicals and small molecules were also compared. Eighteen EU-RMPs (nine biologicals and nine small molecules) given a positive decision regarding the marketing application by the Committee for Medicinal Products for Human Use (CHMP) between November 2005 and May 2007 were included. Classification of the safety concerns, as either important identified risks, important potential risks, and important missing information, was studied as a proxy for the knowledge on the safety profile at the moment of marketing. For PASS

several characteristics were studied; data source (registry, population based database, sponsor-owned clinical trial database), source of study population to be included in PASS, and comprehensiveness of study protocol (full protocol, limited protocol, study synopsis, short description, commitment without further information). A comparison of the classification of the safety concerns, showed that, compared to small molecules, safety concerns for biologicals were less frequently classified as important identified risks (relative risk [RR] 0.6; 95% confidence interval [95% CI] 0.3-1.0] and more frequently as important missing information (RR 1.6; 95% CI 1.0-2.7). A total of 47 PASS were proposed for the 18 products included; 31 for biologicals and 16 for small molecules. Compared with studies proposed in population-based databases (4 for biologicals, 8 for small molecules), studies in registries (18 for biologicals, 4 for small molecules) were more frequently proposed for biologicals than for small molecules (RR 2.5; 95% CI 1.1- 5.7). About 60% of the proposed PASS will (partly) include EU inhabitants. No full study protocols were submitted; 26% involved a limited study protocol, 33% a study synopsis, 37% a short description and 4% a commitment without further information. We concluded that submission of incomplete study protocols precludes an adequate scientific assessment by the regulatory authorities and emphasises the need for more complete study proposals to be submitted earlier on in the evaluation period. Studying non-EU populations may give rise to difficulties with generalisability of the results to the EU due to differences in patient characteristics, differences in the indication for the medicine and different healthcare systems. In addition, differences in the characteristics between biologicals and small molecules, e.g. in the data source proposed, support the need for individualised PASS depending on the type of drug.

Chapter 2.2 and *2.3* studied safety-related regulatory actions issued in the post-marketing setting with regard to the nature, frequency, and timing. Safety-related regulatory actions were defined as 1) dear healthcare professional letters (United States [US]) and direct healthcare professional communications (EU), 2) black box warnings (US), and 3) safety-related market withdrawals (US and EU).

In *chapter 2.2* all biologicals approved in the US and/ or the EU between January 1995 and June 2007 were studied. Vaccines, allergenic products, and products for further manufacture and transfusion purposes were excluded. For the 174 biologicals included in the study 82 safety-related regulatory actions (no safety-related market withdrawals) were issued between January 1995 and June 2008 for 41 biologicals. The probability of a first safety-related regulatory action, derived from Kaplan-Meier analyses, was 14% (95% CI 9%-19%) 3 years after approval and 29% (95% CI 20%-37%) 10 years after approval. Biologicals first in class to obtain approval had a higher risk for a first safety-related regulatory action compared with later approved products in that class (hazard ratio 3.7; 95% CI 1.5-9.5). Warnings mostly concerned the system organ classes general disorders and administration site conditions, infections and infestations, immune system disorders and neoplasms benign, malignant, and unspecified. We concluded that the nature of safety problems identified after approval for biologicals is often related to the immunomodulatory effect (infections). Because the biologicals first to be approved in a class were more likely to be subjected to regulatory action, close monitoring of these biologicals is recommended.

In the study presented in *chapter 2.3* all 95 orphan drugs approved between January 2000 and December 2007 in the US and/ -or the EU were included. Sixteen safety-related regulatory actions (no safety-related market withdrawals) were issued between January 2000 and June 2008 for 10 orphan drugs. The probability of a first safety-related regulatory action for orphan drugs was 20.3% after 8 years of follow-up. Biological orphan drugs had a non-significant higher risk for a first safety-related regulatory action as compared to small molecule orphan drugs (RR 1.7; 95% CI 0.5-5.5). Orphan drugs approved by accelerated approval (RR 3.3; 95% CI 1.1-10.4), oncological products (RR 7.8; 95% CI 1.0-63.8) and products for gastrointestinal and metabolism indications (RR 10.4; 95% CI 1.2-87.3) had a higher risk for a first safety-related regulatory action. It was concluded that the probability of a first safety-related regulatory action for an orphan drug was slightly lower than that reported in the literature and in *chapter 2.2*. However, detection of safety issues may be complicated by the limited experience with orphan drugs in practical use due to the low prevalences of the diseases they are used for. Doctors and pharmacists should therefore be vigilant with regard to the occurrence of a safety-related issue for orphan drugs.

Evaluation and classification of adverse drug reactions in the clinical risk management of biologicals

Chapter 3 focuses on spontaneous adverse drug reactions (ADRs) for biologicals collected during use in the clinical setting and reported to the WHO-ADR database VigiBase.

In *chapter 3.1* we aimed to map the safety profile of biologicals as compared with all other drugs (reference group). Within the group of biologicals, mechanistic classes were compared: antibodies, cytokines, enzymes, growth factors, hormones (reference group), interferons, receptors and other/ various biologicals. Vaccines were not included in the analysis. Suspected ADRs were classified according to MedDRA® at the System Organ Class (SOC) level. Between January 1995 and December 2008 a total of 191,004 case reports containing 546,474 suspected ADRs were reported for 62 different biologicals, and 2,556,209 case reports containing 8,761,522 suspected ADRs were reported for all other drugs (the reference group). It was found that two-thirds of all suspected ADRs reported for biologicals were reported for five active substances: etanercept (20.3%), interferon-β-1a (15.6%), infliximab (11.6%), teriparatide (10.7%) and adalimumab (9.0%). Comparison of the safety profile of biologicals and the reference group showed that suspected ADRs for biologicals were more frequently reported in the SOCs 'Infections and infestations' (proportional reporting ratio [PRR] 4.5), 'Surgical and medical procedures' (PRR 2.4) and 'Neoplasms benign, malignant and unspecified' (PRR 2.1), and less frequently reported in the SOCs 'Psychiatric disorders' (PRR 0.4), 'Vascular disorders' (PRR 0.4) and 'Pregnancy, puerperium and perinatal conditions' (PRR 0.4). Regarding the differences in safety profile between various mechanistic classes of biologicals, compared with hormones (reference group), 'Infections and infestations' were more frequently reported for receptors and antibodies. 'Neoplasms benign, malignant and unspecified' were more frequently reported for antibodies, cytokines, interferons and receptors, and less frequently for enzymes as compared with the reference group.

Chapter 6

It was concluded that since five biologicals comprise two-thirds of the suspected ADRs reported for biologicals, this might distort the relation found between a specific biological and a specific adverse event in case of quantitative signal detection. Therefore the choice of a reference group to be used in case of quantitative signal detection should be considered very carefully. This study confirmed that biologicals have a different safety profile compared with all other drugs in the database and, within the group of biologicals, differences exist between mechanistic classes. Infections are, for example, frequently reported for receptors and antibodies, which often have an immune compromising effect. Such predictable safety issues should be specifically studied by preregistration clinical trials and/or targeted pharmacovigilance. In addition, since not all adverse reactions can be predicted or detected during development, spontaneous reporting remains an important tool for the early detection of signals.

In *chapter 3.2,* VigiBase was used to qualify and quantify how spontaneously reported ADRs fit a new classification system for ADRs of the biological agents cytokines, antibodies, and fusion proteins. The new classification system for ADRs of biologicals was proposed because of expected limitations of the traditional classification system of ADRs in type A and B ADRs. In this new classification system for ADRs of biologicals five categories are proposed: high cytokine and cytokine release syndrome (type α), hypersensitivity reactions to biological agents (type β), immune/ cytokine imbalance syndromes (type γ), cross-reactivity (type δ), and non-immunological side-effects (type ε). In total, 17 type β, 21 type γ, and 24 type ε ADRs were selected, for which Reporting Odds Ratios (ROR) were calculated (small molecules as reference). Within VigiBase no ADRs could be identified in the class δ and only two ADRs in class α. These classes were therefore not included in this study. To study the correlation between pairs of ADRs cluster analysis and pair-wise dissimilarities were used. Cluster analysis resulted in 7 clusters; *cluster 1* contained 2 β and 22 ε ADRs, *cluster 2* contained 1 β, 13 γ, 2 ε, *cluster 3* contained 2 β and 4 γ ADRs, *cluster 4* contained 3 γ ADRs, and *clusters 5, 6, and 7* contained 5, 2, and 4 β ADRs, respectively. Calculated pair-wise dissimilarities for the ADRs classified according to the new classification system showed differentiation between the type β and γ versus the type ε ADRs and correlation of ADRs related to the stage of the hypersensitivity reaction (infusion related relations, immediate-type hypersensitivity and delayed type hypersensitivity). We concluded that the proposed classification system seems to be valid for differentiation between the immunological β and γ ADRs and the non-immunological ε ADRs. One of the interest in pharmacovigilance is the identification of certain ADRs based on a combination of search terms, e.g. ADRs indicative of a hypersensitivity reaction. Combination of ADRs related to type γ ADRs might be useful for signal detection. Within the β ADRs, related ADRs showed correlation based on the phase of the hypersensitivity reaction.

Risk factors and early warning markers in the clinical risk management of biologicals: rituximab as a learning case

Chapter 4 focuses on risk factors and early warning markers for the early identification of patients at risk for a certain ADR. Rituximab was used as a learning case in the studies presented in this chapter. Rituximab-users were selected from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medication orders, and laboratory tests for all patients treated at the University Medical Center Utrecht since 2004.

Chapter 4.1 studied the incidence and risk factors for invasive Aspergillosis (IA) in patients treated with rituximab for haematological malignancies between 2005 and 2008. IA is a major cause of death in oncology patients and, although a causal role of rituximab in the development of IA has not been established, two cases reports and one epidemiological study suggest a role of rituximab. For IA it is known that early diagnosis and subsequent early initiation of therapy improves outcome underlining the need for the identification of risk factors. A total of 104 patients had been treated with rituximab in the study period of which seven patients were diagnosed with probable IA (cumulative incidence: 6.7%). A nested case-control study was performed in which only the patients who had undergone an allogeneic stem cell transplantation were included. Within the nested case-control analysis 3 controls were sampled for each case. It was shown that patients who developed IA had been treated more frequently with a cumulative dose of rituximab of 1500 mg or more (odds ratio [OR] 25.5; 95% CI 2.4-275.7) and had more frequently been diagnosed with another fungal infection in the 30 days before the diagnosis of IA (OR 15.0; 95% CI 1.2-183.6). All cases diagnosed with a fungal infection were diagnosed with a Candida infection. It was concluded that the cumulative incidence seemed to be comparable to incidences found in other studies in patients with haematological malignancies. Patients treated with a high cumulative dose of rituximab and patients diagnosed with fungal infections in the 30 days before the diagnosis of IA were at an increased risk for IA. These findings stress the importance of measures to minimise the risk for IA, such as activities to minimise the exposure to potentially invasive fungus and preventive treatment with anti-fungal therapy.

In *chapter 4.2* the incidence of rituximab-induced thrombocytopenia as well as risk factors and early warning markers were studied. Thrombocytopenia is a known ADR of rituximab but information on incidence, risk factors and early warning markers is limited. Ninety patients were eligible for inclusion in the present study of whom 27 patients developed thrombocytopenia (cumulative incidence: 30%) within the 30 days after administration of rituximab. A total of 18 patients developed grade 3/4 thrombocytopenia (cumulative incidence: 20%). Risk factors and early warning markers were studied in a nested case-control analysis, in which all patients with thrombocytopenia (cases) were compared with all patients without thrombocytopenia (controls). A multivariate model identified a relatively low platelet count before administration of rituximab (217.5 vs. 324.4x10^9 platelets/L; p=0.011) and, although not statistically significant, use of rituximab within the oncology indications versus the auto-immune indications (OR 4.7; 95% CI 1.0-23.3) and a high platelet distribution width (PDW) before administration of rituximab (16.1 vs. 15.8; p=0.051), as independent risk factors for the development of thrombocytopenia.

With regard to the potential early warning markers after administration of rituximab, patients developing thrombocytopenia had an average platelet count below $200\text{x}10^9$ platelets/L in the week preceding the first measurement of thrombocytopenia whereas the average platelet count was above $200\text{x}10^9$ platelets/L for patients who did not develop thrombocytopenia. It was concluded that the incidence of thrombocytopenia found in the present study was higher than identified during the clinical trials. Healthcare professionals should therefore consider thrombocytopenia as a relevant ADR during treatment with rituximab. More frequent monitoring of the thrombocyte count is especially advised in patients treated with rituximab in the oncology indication and/ or with a relatively low platelet count and high PDW before start of rituximab therapy and a relatively low platelet count after treatment with rituximab.

Discussion

In chapter 6, the general discussion, four topics are discussed in a broader context. These topics are: (1) Exposure and outcome assessment of biologicals, (2) Datasources for the conduct of pharmacoepidemiological studies of biologicals, (3) Assessment of the hazard function of biologicals, and (4) susceptibility for ADRs of patients treated with biologicals. We give recommendations for regulatory and clinical practice.

In conclusion, this thesis discusses the pharmacovigilance of biologicals with a strong focus on regulatory and clinical practice. Information on the characteristics of biologicals and their underlying ADR profile is important in regulatory and clinical risk management. Within regulatory risk management, the approach is changing from a reactive towards a more proactive approach and knowledge obtained with other biologicals should be used during the assessment of new biological agents. For clinical risk management, information on the safety profile of biologicals and use of risk factors and early warning markers can lead to a safer use of drugs in clinical practice and minimise the risk for the patient.

Samenvatting

Biologicals worden door de European Medicines Agency (EMA), het Europese genees-middelenbureau, gedefinieerd als producten waarbij een (gedeelte van een) organisme wordt gebruikt voor de productie van het uiteindelijke geneesmiddel. Dit betekent dat geneesmiddelen die geëxtraheerd worden uit menselijk of dierlijk materiaal tot de biologicals worden gerekend. Een voorbeeld hiervan zijn stollingsfactoren die geëxtraheerd worden uit humaan plasma. De meeste biologicals worden echter geproduceerd met behulp van recombinant DNA technologieën. Hiervoor worden cellijnen gebruikt van bijvoorbeeld hamsters of gisten die gemodificeerd zijn en daardoor in staat zijn tot de productie van bepaalde eiwitten. Een bekend biological is insuline dat al vele jaren wordt gebruikt voor de behandeling van diabetes mellitus. In de jaren twintig van de vorige eeuw werd insuline voor het eerst geëxtraheerd vanuit de alvleesklier van varkens. Sinds de introductie van de recombinant DNA technologieën in de jaren tachtig van de vorige eeuw wordt insuline op grote schaal geproduceerd met behulp van deze techniek. Naast insuline is er momenteel een groot aantal geneesmiddelen beschikbaar die tot de biologicals behoren. Biologicals nemen op dit moment onder andere een belangrijke plaats in bij de behandeling van patiënten met kanker en auto-immuunziekten. In vergelijking met de traditionele, chemisch gesynthetiseerde geneesmiddelen, zoals bijvoorbeeld parace-tamol, hebben biologicals specifieke karakteristieken die van invloed kunnen zijn op hun veiligheid. Waar de chemisch gesynthetiseerde geneesmiddelen vaak een relatief eenvou-dig productieproces hebben, hebben biologicals vaak een zeer complex productieproces. Daarnaast hebben biologicals een groot risico voor immunogeniciteit. Immunogeniciteit houdt in dat er antilichamen worden geproduceerd als reactie op de toediening van een biological. Het biological kan verschillen van het humaan aanwezige eiwit waardoor het lichaam het toegediende biological als lichaamsvreemd beschouwt en een immuunreactie op gang brengt. In veel gevallen is de vorming van antilichamen niet klinisch relevant. De antilichamen kunnen het effect van het toegediende biological echter neutraliseren waardoor de effectiviteit afneemt. Er zijn gevallen beschreven waar de vorming van an-tilichamen tot ernstige veiligheidsproblemen hebben geleid. De voorspelbaarheid van preklinische onderzoeken (dierstudies) voor de klinische praktijk is tevens beperkt voor biologicals. Bepaalde biologicals gedragen zich namelijk anders bij de verschillende or-ganismen en dieren vormen vaak antilichamen aangezien een menselijk eiwit als li-chaamsvreemd wordt beschouwd. Bijwerkingen van biologicals kunnen tenslotte vaak worden gerelateerd aan een versterkt farmacologisch effect.

Het is bekend dat onderzoeken uitgevoerd voor de registratie van een geneesmiddel be-paalde beperkingen hebben ten aanzien van het identificeren van (zeldzame) bijwerkin-gen. Deze beperkingen bestaan onder andere uit een relatief kleine groep patiënten die bovendien streng geselecteerd is. Gegevens over bijwerkingen geïdentificeerd na regi-stratie van een geneesmiddel vormt daardoor een belangrijke aanvulling op de informatie zoals verzameld tijdens de onderzoeken uitgevoerd voor registratie. De farmacovigilantie (geneesmiddelenbewaking) is de wetenschap die zich hiermee bezig houdt. Farmacovi-gilantie wordt gedefinieerd als de wetenschap die zich bezighoudt met de detectie, be-oordeling en preventie van bijwerkingen van geneesmiddelen nadat deze zijn toegelaten

tot de markt. De methodes die worden gebruikt voor de detectie van bijwerkingen kunnen grofweg in twee methoden worden onderverdeeld: 1) afwachtend, gebaseerd op de verzameling van bijwerkingen die spontaan gemeld worden door artsen, apothekers en patiënten en 2) de meer proactieve benadering waarbij specifieke onderzoeken worden opgezet voor de identificatie en kwantificering van bijwerkingen. De proactieve benadering is in de Europese Unie (EU) in 2005 geïmplementeerd met de verplichte indiening van een zogenaamd EU-Risk Management Plan (EU-RMP) voor de registratieaanvraag van nieuwe geneesmiddelen, inclusief biologicals. In het EU-RMP worden onder andere aanvullende activiteiten beschreven om het bijwerkingenprofiel verder in kaart te brengen op het moment dat het geneesmiddel op de markt komt. Naast het verzamelen van spontane meldingen van bijwerkingen kunnen aanvullende activiteiten bestaan uit epidemiologische onderzoeken (zogenaamde post-authorisation safety studies [PASS]) waarbij een groep patiënten wordt gevolgd in de tijd en bijwerkingen worden verzameld. Echter, zoals eerder beschreven verschillen biologicals van de chemisch gesynthetiseerde geneesmiddelen en deze verschillen kunnen ook tot uitdrukking komen in de farmacovigilantie van deze producten. In dit proefschrift is de farmacovigilantie van de biologicals bestudeerd vanuit een regulatoir en een klinisch perspectief.

Regulatoire activiteiten in het beheersen van risico's van biologicals

In hoofdstuk 2 zijn drie onderzoeken gepresenteerd. Deze onderzoeken richtten zich op de regulatoire activiteiten in de beheersing van de risico's van biologicals. Zoals reeds beschreven in de introductie dienen farmaceutische bedrijven een EU-RMP in te dienen als onderdeel van de registratieaanvraag van onder andere biologicals.

In hoofdstuk 2.1 hebben we de activiteiten bestudeerd die zijn voorgesteld door farmaceutische bedrijven om het veiligheidsprofiel na registratie verder in kaart te brengen en is de methode die is gebruikt voor PASS bestudeerd. Daarnaast zijn problemen met PASS geïdentificeerd en zijn voorstellen gedaan om de geïdentificeerde problemen te verbeteren. Binnen dit onderzoek zijn de karakteristieken van biologicals en chemisch gesynthetiseerde geneesmiddelen vergeleken. In totaal zijn achttien EU-RMPs (9 voor biologicals en 9 voor chemisch gesynthetiseerde geneesmiddelen) bestudeerd. De EU-RMPs waren van producten die goedgekeurd waren door het wetenschappelijk comité voor geneesmiddelen voor humaan gebruik (CHMP) tussen november 2005 en mei 2007. Binnen het EU-RMP worden mogelijke veiligheidsproblemen geclassificeerd als a) belangrijk geïdentificeerd risico (er is een causaal verband tussen het veiligheidsprobleem en het geneesmiddel), b) belangrijk potentieel risico (er bestaat potentieel een veiligheidsprobleem op basis van bijvoorbeeld het werkingsmechanisme van het geneesmiddel) en c) belangrijke informatie die ontbreekt en het veiligheidsprofiel van het geneesmiddel kan beïnvloeden. De classificatie van de mogelijke veiligheidsproblemen geeft inzicht in de kennis van het veiligheidsprofiel op een bepaald moment in de tijd. Een vergelijking van de classificatie van de mogelijke veiligheidsproblemen liet zien dat veiligheidsproblemen voor biologicals minder frequent geclassificeerd waren als belangrijk geïdentificeerd risico (relatief risico [RR] 0.6; 95% betrouwbaarheidsinterval [95% BI] 0.3-1.0) en vaker als belangrijke informatie die ontbrak (RR 1.6; 95% BI 1.0-2.7) in vergelijking met de chemisch gesynthetiseerde geneesmiddelen. Voor PASS zijn verschillende karak-

teristieken bestudeerd: de database gebruikt voor de onderzoeken (registers [waarbij patiënten met een bepaalde ziekte en/-of behandeling wordt geïncludeerd], populatie gebaseerde database [waarbij patiënten in een bepaald gebied automatisch worden geïncludeerd], een database die gegevens van klinisch onderzoek bevat), landen in de wereld van waaruit patiënten zijn geïncludeerd in PASS en volledigheid van het studieprotocol van PASS (volledig studieprotocol, beperkt studieprotocol, studie synopsis, korte beschrijving van het onderzoek, toezegging zonder verdere informatie). In totaal werden 47 PASS beschreven in de 18 EU-RMPs; 31 voor biologicals en 16 voor chemisch gesynthetiseerde geneesmiddelen. PASS voor biologicals werden vaker voorgesteld om te worden uitgevoerd in registers in vergelijking met PASS voor chemisch gesynthetiseerde geneesmiddelen die frequenter werden uitgevoerd in populatie gebaseerde databases. Ongeveer 60% van de PASS includeren inwoners van de EU. In 40% van de PASS worden dus geen inwoners van de EU meegenomen wat kan leiden tot problemen in de generaliseerbaarheid van de resultaten verkregen met deze onderzoeken naar de populatie binnen de EU. De beperkte generaliseerbaarheid kan veroorzaakt worden door verschillen tussen patiënten in verschillende werelddelen, verschillen in goedgekeurde indicaties en verschillen in de gezondheidszorg. Met betrekking tot de studieprotocollen is gevonden dat er geen volledige studieprotocollen zijn ingediend; 26% betrof een beperkt studieprotocol; 33% een synopsis van het onderzoek, 37% een korte beschrijving en 4% een toezegging zonder verdere informatie. Op basis van dit onderzoek is geconcludeerd dat indiening van incomplete studieprotocollen bij de regulatoire autoriteiten een afdoende wetenschappelijke beoordeling stoort. Het indienen van meer volledige studieprotocollen is daarom noodzakelijk. Daarnaast zijn verschillen gezien tussen biologicals en de chemisch gesynthetiseerde geneesmiddelen met betrekking tot de kennis omtrent het veiligheidsprofiel op het moment van registratie en de gebruikte database. Dit benadrukt de noodzaak dat PASS product specifiek moet worden opgezet.

Hoofdstuk 2.2 en 2.3 richten zich op het ingrijpen door regulatoire autoriteiten in verband met veiligheid van geneesmiddelen. In deze hoofdstukken is gekeken naar het type bijwerking, de frequentie van ingrijpen door de regulatoire autoriteiten en de tijd tussen goedkeuring van de registratie aanvraag en het ingrijpen door de regulatoire autoriteiten. De volgende maatregelen zijn meegenomen: 1) brieven die gestuurd zijn aan artsen en apothekers in de Verenigde Staten (VS) en de EU, 2) black box warnings in de VS (black box warnings zijn veiligheidswaarschuwingen die bovenaan de bijsluiter worden vermeld in een zwart kader) en 3) terugtrekking van de markt van het geneesmiddel in de VS of de EU als gevolg van één of meerdere bijwerkingen.

In hoofdstuk 2.2 zijn alle in de VS en de EU tussen januari 1995 en juni 2007 goedgekeurde biologicals meegenomen. Vaccins, allergenen en producten die voor verdere bewerking en transfusie worden gebruikt zijn geëxcludeerd. Voor het totaal aantal van 174 goedgekeurde biologicals werd er tussen januari 1995 en juni 2008 82 maal ingegrepen door de regulatoire autoriteiten. Dit betrof 41 verschillende biologicals. De kans op een eerste regulatoir ingrijpen was 3 jaar na registratie 14% (95% BI 9%-19%) voor biologicals en 10 jaar na registratie 29% (95% BI 20%-37%). Regulatoir ingrijpen was vaker nodig voor biologicals die als eerste zijn goedgekeurd in een Anatomische Therapeutisch Chemische (ATC) subklasse in vergelijking tot biologicals die later zijn goedgekeurd

(hazard ratio 3.7; 95% BI 1.5-9.5). Dit onderlijnt de noodzaak dat biologicals die als eerste worden goedgekeurd in een klasse nauwlettend in de gaten moeten worden gehouden na registratie. De veiligheidswaarschuwingen betroffen voornamelijk de categorieën Algemene waarschuwingen en toedieningsplaatsstoornissen, Infecties en parasitaire aandoeningen, Immuunsysteemaandoeningen en Neoplasmata, benigne, maligne en niet gespecificeerd (inclusief cysten en poliepen). Op basis van deze resultaten hebben we geconcludeerd dat veiligheidswaarschuwingen voor biologicals vaak gerelateerd zijn aan het farmacologische werkingsmechanisme (bijvoorbeeld infecties).

In het onderzoek beschreven in hoofdstuk 2.3 zijn alle 95 tussen januari 2000 en december 2008 goegekeurde geneesmiddelen voor de behandeling van zeldzame aandoeningen – zogenaamde weesgeneesmiddelen – meegenomen. In totaal was er tussen januari 2000 en juni 2009 16 keer regulatoir ingrijpen nodig voor tien verschillende geneesmiddelen. De kans op een eerste regulatoir ingrijpen was 8 jaar na goedkeuring 20.3%. In dit onderzoek zijn biologicals vergeleken met chemisch gesynthetiseerde geneesmiddelen. Biologicals hebben een niet-significant hoger risico op regulatoir ingrijpen (relatief risico [RR] 1.7; 95% BI 0.5-5.5). Geneesmiddelen goedgekeurd via een versnelde registratie procedure (RR 3.3; 95% BI 1.1-10.4) alsmede geneesmiddelen goedgekeurd voor oncologische indicaties (RR 7.8; 95% BI 1.0-63.8) en voor indicaties gerelateerd aan het maag-darmstelsel en het metabolisme (RR 10.4; 95% BI 1.-87.3) hebben een hoger risico op regulatoir ingrijpen. Op basis van deze gegevens is geconcludeerd dat geneesmiddelen voor zeldzame aandoeningen een lagere kans hadden op regulatoir ingrijpen in vergelijking tot datgene beschreven in de literatuur en in hoofdstuk 2.2 van dit proefschrift. Echter, identificatie van veiligheidsproblemen is waarschijnlijk lastiger voor geneesmiddelen die goedgekeurd zijn voor zeldzame aandoeningen vanwege de zeldzaamheid van de aandoeningen en daarmee samenhangend een beperkt gebruik waardoor zeldzame bijwerkingen minder snel kunnen worden ontdekt. Artsen en apothekers dienen daarom te waken voor veiligheidsproblemen die gerelateerd zijn aan deze groep geneesmiddelen.

Evaluatie en classificatie van bijwerkingen van biologicals in de klinische praktijk

Hoofdstuk 3 richt zich op spontane meldingen van bijwerkingen van biologicals zoals verzameld gedurende gebruik in de klinische praktijk. Hiervoor is de database van de WHO, VigiBase, gebruikt waarin wereldwijd spontane bijwerkingen van geneesmiddelen worden verzameld.

In hoofdstuk 3.1 hebben we getracht het bijwerkingenprofiel van de biologicals in kaart te brengen en dit te vergelijken met het profiel van de chemisch gesynthetiseerde geneesmiddelen. Daarnaast zijn verschillende groepen van biologicals onderling vergeleken. Hiervoor zijn biologicals geclassificeerd op basis van eigenschappen gerelateerd aan hun structuur en/ of werkingsmechanisme. Vaccines zijn niet meegenomen in dit onderzoek. Tussen januari 1995 en december 2008 zijn 191.004 rapporten van bijwerkingen gerapporteerd voor 62 biologicals. Dit betrof 546.474 verschillende bijwerkingen. In de referentiegroep (voornamelijk betaande uit chemisch gesynthetiseerde geneesmiddelen) zijn 2.556.209 rapporten van bijwerkingen ontvangen. Dit betrof 8.761.522 verschillende bijwerkingen. Voor de biologicals is gevonden dat 2/3 van alle bijwerkingen gemeld zijn

voor slechts vijf actieve bestanddelen: etanercept, interferon-β-1a, infliximab, teriparatide en adalimumab. Vergelijking van het bijwerkingenprofiel van de biologicals met de referentiegroep laat zien dat bijwerkingen van biologicals die geïdentificeerd zijn gedurende gebruik in de klinische praktijk voornamelijk gerelateerd waren aan 'Infecties en parasitaire aandoeningen' (proportional reporting ratio [PRR] 4.5), 'Chirurgische en medische verrichtingen' (PRR 2.4) en en Neoplasmata, benigne, maligne en niet gespecificeerd (inclusief cysten en poliepen) (PRR 2.1). Bijwerkingen van biologicals die geïdentificeerd zijn tijdens gebruik in de klinische praktijk betroffen minder vaak bijwerkingen gerelateerd aan 'Psychische aandoeningen' (PRR 0.4), 'Bloedvataandoeningen' (PRR 0.4) en 'Zwangerschap, perinatale periode en puerperium' (PRR 0.4). Een vergelijking binnen de groep van de biologicals liet zien dat ook binnen de biologicals verschillen bestaan in het bijwerkingenprofiel. Er werd onder andere gevonden dat bijwerkingen gerelateerd aan 'Infecties en parasitaire aandoeningen' frequenter gerapporteerd zijn voor antilichamen en receptoren in vergelijking met de hormonen. We hebben geconcludeerd dat er voor de kwantitatieve signaaldetectie zorgvuldig moet worden gekeken naar de referentiegroep aangezien 2/3 van alle meldingen voor de biologicals slechts vijf actieve bestanddelen betrof. Inclusie van deze vijf biologicals in de referentiegroep kan mogelijke associaties ernstig verstoren. Dit onderzoek liet nogmaals zien dat biologicals een ander bijwerkingenprofiel hebben dan de chemisch gesynthetiseerde geneesmiddelen en dat er ook binnen de groep van de biologicals verschillen bestaan. Dit onderzoek liet bijvoorbeeld zien dat infecties frequenter zijn gemeld voor biologicals met een immuunsuppressieve werking. Voorspellen van het bijwerkingenprofiel aan de hand van het werkingsmechanisme is belangrijk voor biologicals. Op basis daarvan kunnen bepaalde bijwerkingen specifiek in de gaten worden gehouden na registratie. Echter, aangezien niet alle bijwerkingen kunnen worden voorspeld blijft spontane rapportage van bijwerkingen noodzakelijk.

In hoofdstuk 3.2 is VigiBase gebruikt om een nieuw classificatiesysteem van bijwerkingen specifiek voor drie groepen biologicals – cytokines, antilichamen en fusie-eiwitten – te kwalificeren en te kwantificeren. Traditioneel worden bijwerkingen ingedeeld in type A (bijwerkingen gerelateerd aan het werkingsmechanisme en bijwerkingen die dosisafhankelijk zijn) en type B (zeldzame, niet voorspelbare bijwerkingen zoals bijvoorbeeld immuunreacties) bijwerkingen. Aan de toepasbaarheid van dit classificatiesysteem voor biologicals wordt getwijfeld aangezien immuunreacties, bijvoorbeeld, gerelateerd kunnen worden aan de karakteristieken van biologicals en daardoor wellicht als type A bijwerking geclassificeerd dienen te worden. In het nieuwe classificatiesysteem voor biologicals worden vijf groepen bijwerkingen onderscheiden: bijwerkingen gerelateerd aan een hoog aantal cytokines en cytokine release syndrome (type α), overgevoeligheidsreacties (type β), bijwerkingen gerelateerd aan het immuunsysteem en/ of een disbalans in het cytokine gehalte (type γ), cross-reactiviteit met een receptor die zich op een andere plaats in het lichaam bevindt en daar een bijwerking veroorzaakt (type δ) en bijwerkingen die niet gerelateerd zijn aan immunologische reacties (type ε). Binnen VigiBase zijn 17 type β, 21 type γ en 24 type ε bijwerkingen geselecteerd. Er konden slechts twee type α en geen type δ bijwerkingen worden geïdentificeerd. Deze groepen zijn daarom niet meegenomen. Voor elke combinatie van een bijwerking en een biological is een reporting odds ratio

(ROR) berekend. Op basis van de RORs zijn clusters van bijwerkingen geïdentificeerd. Deze analyse is gebaseerd op de aanname dat gerelateerde bijwerkingen een vergelijkbaar rapportageprofiel hebben en dientengevolge een vergelijkbare ROR, waardoor deze samen worden geclusterd. Hierdoor kan een combinatie van gerelateerde bijwerkingen worden vastgestelde. De combinatie van bijwerkingen is zinvol in het geval van signaal-detectie. De clusteranalyse resulteerde in zeven clusters; cluster 1 bestond uit 2 β en 22 ε bijwerkingen, cluster 2 bevatte 1 β, 13 γ en 2 ε bijwerkingen, cluster 3 bevatte 2 β en 4 γ bijwerkingen, cluster 4 bevatte 3 γ bijwerkingen en clusters 5, 6, en 7 bestonden respectievelijk uit 5, 2 en 4 type β bijwerkingen. Naast de clusteranalyse is de relatie tussen bijwerkingen ook kwantitatief bepaald. Hiervoor is voor elke combinatie van twee bijwerkingen een waarde uitgerekend die een maat is voor de (on)gelijkheid van de bijwerkingen. Op basis van deze berekeningen hebben we bijvoorbeeld laten zien dat de immunologische bijwerkingen geclassificeerd als type β en γ bijwerkingen onderling een meer vergelijkbaar rapportageprofiel hebben dan de type ε bijwerkingen die alle niet-immunologische bijwerkingen omvatten. Daarnaast hebben binnen de type β bijwerkingen, reacties die samenhangen met hetzelfde stadium van de overgevoeligheidsreactie (type I en type IV overgevoeligheidsreacties) een vergelijkbaar rapportageprofiel. Op basis van deze resultaten is geconcludeerd dat het voorgestelde classificatiesysteem voor bijwerkingen van biologicals in staat is te differentiëren tussen de immunologische type β en γ bijwerkingen en de niet- immunologische type ε bijwerkingen. Binnen de type β bijwerkingen toonden gerelateerde bijwerkingen op basis van het stadium van de overgevoeligheidsreacties een vergelijkbaar rapportageprofiel.

Beheersing van risico's van biologicals in de klinische praktijk: het voorbeeld van rituximab

De onderzoeken beschreven in hoofdstuk 4 richten zich op het identificeren van risicofactoren en markers waarmee patiënten met een verhoogd risico op een bepaalde bijwerking in een vroeg stadium kunnen worden geïdentificeerd. In de onderzoeken beschreven in dit hoofdstuk wordt gebruik gemaakt van een groep patiënten die behandeld zijn met rituximab. Gebruikers van rituximab zijn geïdentificeerd in de Utrecht Patient Oriented Database (UPOD). UPOD is tot stand gekomen door het koppelen van databases waardoor complete informatie beschikbaar is met betrekking tot patiëntkarakteristieken, diagnoses, medicatiegebruik en laboratoriumgegevens. UPOD omvat deze data voor alle patiënten die behandeld zijn in het Universitair Medisch Centrum Utrecht sinds 2004.

In hoofdstuk 4.1 hebben we een onderzoek gepresenteerd waarin we de incidentie en risicofactoren hebben bestudeerd voor het optreden van invasieve Aspergillose (IA) – een ernstige schimmelinfectie – bij patiënten die behandeld zijn met rituximab tussen 2005 en 2008 voor hematologische maligniteiten. IA is een belangrijke doodsoorzaak in patiënten met maligniteiten. Hoewel een specifieke rol voor rituximab nog niet is aangetoond, suggereren twee patiëntbeschrijvingen en één epidemiologisch onderzoek een rol voor rituximab. Het is bekend dat een vroege diagnose en dientengevolge een vroege behandeling de uitkomst voor de patiënt sterk verbetert. Dit benadrukt het belang van een vroege identificatie van patiënten met een verhoogd risico op IA. In totaal zijn 104 patiënten behandeld met rituximab voor hematologische maligniteiten tussen 2005 en 2008

waarvan zeven zijn gediagnosticeerd met IA (cumulatieve incidentie: 6.7%). Binnen deze groep is een case-control onderzoek uitgevoerd waarin alleen de patiënten die een allogene stamceltransplantatie hadden ondergaan zijn meegenomen. We hebben gevonden dat patiënten die IA ontwikkelden behandeld waren met een hogere cumulatieve dosis van rituximab (<1500 mg versus ≥1500 mg; odds ratio [OR] 25.5; 95% BI 2.4-275.7) en dat deze patiënten frequenter waren gediagnosticeerd met andere schimmelinfecties in de 30 dagen voor de diagnose IA (OR 15.0; 95% BI 1.2-183.6). Op basis van deze data hebben we geconcludeerd dat de cumulatieve incidenctie in deze groep rituximabgebruikers vergelijkbaar is met de incidenties zoals gevonden in andere onderzoeken waarin patiënten met hematologische maligniteiten zijn bestudeerd. Patiënten die behandeld zijn met een hogere cumulatieve dosis rituximab alsmede patiënten die gediagnosticeerd zijn met schimmelinfecties in de 30 dagen voor de diagnose IA hebben een verhoogd risico op het ontwikkelen van IA.

In hoofdstuk 4.2 hebben we de incidentie van rituximab-geïnduceerde trombocytopenie – te lage hoeveelheid bloedplaatjes – bestudeerd alsmede risicofactoren en bloedwaarden die kunnen worden gebruikt voor een vroege identificatie van patiënten met een verhoogd risico op trombocytopenie. Trombocytopenie is een bekende bijwerking van rituximab maar informatie met betrekking tot incidentie, risicofactoren en hematologische markers is beperkt. Negentig patiënten die behandeld zijn met rituximab tussen 2005 en 2009 zijn meegenomen in dit onderzoek waarvan 27 patiënten trombocytopenie ontwikkelden (cumulatieve incidentie: 30%) binnen 30 dagen na toediening van de rituximab en 18 patiënten graad 3/4 trombocytopenie ontwikkelden. Patiënten met trombocytopenie zijn vergeleken met patiënten die geen trombocytopenie ontwikkelden. In deze analyse is een relatief laag aantal bloedplaatjes voor toediening van rituximab (217.5 versus 324.4x10^9 bloedplaatjes/L; p=0.011) geïdentificeerd als risicofactor voor trombocytopenie. Daarnaast zijn behandeling met rituximab voor maligniteiten in vergelijking met behandeling voor auto-immuunziekten (OR 4.7; 95% BI: 1.0-23.3) en een grotere spreiding in het volume van de trombocyten voor toediening van rituximab (16.1 versus 15.8; p=0.051) geïdentificeerd als risicofactoren voor trombocytopenie. Daarnaast zagen wij dat patiënten die trombocytopenie ontwikkelden een lager aantal trombocyten hadden in de week voor dat de diagnose trombocytopenie werd gesteld in vergelijking tot patiënten die geen trombocytopenie ontwikkelden. We hebben geconcludeerd dat trombocytopenie in de klinische praktijk vaker voorkomt dan in klinische onderzoeken die uitgevoerd zijn voor registratie van rituximab. Artsen en apothekers dienen derhalve rekening te houden met trombocytopenie als een relevante bijwerking na toediening van rituximab. Frequentere meting van het aantal trombocyten wordt aangeraden voor patiënten die behandeld worden met rituximab voor maligniteiten en/ of patiënten met een relatief laag aantal trombocyten voor toediening van rituximab.

Discussie

In hoofdstuk 6 zijn vier onderwerpen besproken in relatie tot de farmacovigilantie en farmacoepidemiologie van biologicals. Daarnaast worden aanbevelingen gedaan voor de regulatoire en klinische praktijk.

Concluderend, we hebben ons in dit proefschrift gericht op de farmacovigilantie van biologicals met een sterke focus op de regulatoire en klinische praktijk. Kennis omtrent het bijwerkingenprofiel en de karakteristieken van de biologicals is belangrijk in beheersing van de risico's in de regulatoire en klinische praktijk. In het regulatoire veld verschuift de insteek meer en meer van een afwachtende naar een proactieve aanpak. Het werkingsmechanisme van een nieuw biological en de daarmee samenhangende verwachte bijwerkingen dienen nauwkeurig te worden beoordeeld voor registratie en dienen na registratie nauwlettend in de gaten te worden gehouden. In de klinische praktijk wordt verwacht dat informatie met betrekking tot de bijwerkingen van biologicals en kennis omtrent patiënten met een verhoogd risico voor een bepaalde bijwerking zal leiden tot een veiliger gebruik van geneesmiddelen en dientengevolge een afname van het risico van een bijwerking voor de patiënt.

Chapter 7

Dankwoord

Dankwoord

Voordat je het weet ben je dit laatste hoofdstuk aan het schrijven en denk je terug aan de afgelopen vier jaar. Een periode waarin ik heel veel heb geleerd en een enorme ontwikkeling door heb mogen maken. Dit komt voor een groot deel door mijn beide promotoren en co-promotoren die mij bleven stimuleren zelf na te blijven denken en de mogelijkheid boden om met veel verschillende mensen samen te werken.

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Chapter 8

List of co-authors

List of co-authors

Affiliations during the conductance of this thesis

Maarten J. ten Berg
Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, the Netherlands

Stella Blackburn
European Medicines Agency, London, United Kingdom

Andries C. Bloem
Department of Immunology, University Medical Center Utrecht, Utrecht, the Netherlands

Tjomme van den Bruggen
Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands

Ivor R. Edwards
WHO Collaborating Centre for International Drug Monitoring, Uppsala Monitoring Centre, Uppsala Sweden

Toine C.G. Egberts
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands
&
Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht, the Netherlands

Frits GmeligMeylin
Department of Immunology, University Medical Center Utrecht, Utrecht, the Netherlands

Harald E. Heemstra
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands

Hubert G.M. Leufkens
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands
&
Medicines Evaluation Board, the Hague, the Netherlands

Aukje K. Mantel-Teeuwisse
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands
&
Medicines Evaluation Board, the Hague, the Netherlands

Ronald H.B. Meijboom
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands
&
WHO Collaborating Centre for International Drug Monitoring, Uppsala Monitoring Centre, Uppsala Sweden

Ingemar Persson
Medical Products Agency, Uppsala, Sweden
&
Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

Huub Schellekens
Division of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands
&
Department of Innovation and Environmental Sciences, Faculty of Geosciences, Utrecht University, Utrecht, the Netherlands

Wouter W. van Solinge
Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands
&
Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, the Netherlands

Sabine M.J.M. Straus
Medicines Evaluation Board, the Hague, the Netherlands
&
Department of Medical Informatics, Erasmus MC, Rotterdam, the Netherlands

Remco L. de Vrueh
Steering Committee on Orphan Drugs, the Hague, the Netherlands

Chapter 9

List of publications

List of publications

Related to this thesis

Giezen TJ, Mantel-Teeuwisse AK, Meijboom RHB, Straus SM, Leufkens HGM, Egberts TCG.
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Chapter 10

About the author

About the author

Thijs Giezen was born on 15 June 1982 in Rotterdam, the Netherlands. In 2000, he completed secondary school (Gymnasium) at the Scholengemeenschap Aquamarijn in Vlaardingen. In 2000, he started studying pharmacy at Utrecht University. As part of his studies he completed a research traineeship at the Prince Henry's Institute, Melbourne, Australia. In 2005, he obtained his Master's degree in pharmacy, followed by his pharmacist degree (PharmD) in 2006.

Thereafter, he worked on the studies described in this thesis at the Division of Pharmacoepidemiology & Clinical Pharmacology of the Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University in affiliation with the Medicines Evaluation Board in the Hague. The PhD studies were combined with a position as a pharmacovigilance assessor at the Pharmacovigilance Department of the Medicines Evaluation Board. During this period he obtained a Master of Science degree in Epidemiology at the Julius Center of the University Medical Center in Utrecht.

End of 2010, he started his training to become a hospital pharmacist at the Department of Clinical Pharmacy at the 'Medisch Spectrum Twente' in Enschede.