Pharmacoepidemiological aspects of drug-induced bleeding

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2015
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ACKNOWLEDGEMENTS

This work was carried out at the Department of Pharmacology at Oslo University Hospital (2007 – 2011), and completed at Center for Psychopharmacology at Diakonhjemmet Hospital (2012 – 2015). The studies in this thesis were inspired/encouraged by the work at RELIS where too many case reports concerned patients who suffered severe bleeding adverse drug reactions.

First of all, I would like to thank my main supervisor, Marianne Kristiansen Kringen, who was also my roommate the years at Ullevål. I am most grateful for your utmost patience with me as the progress with the project was slow due to shift of work places and private obligations. I would thank you for always clear and quick replies and never ending optimism for the project.

Next, I would like to thank Professor Odd Brørs, my co-supervisor, for giving me, an otorhinolaryngologist, the opportunity to change field and learn pharmacology by giving me a position in clinical pharmacology. No matter how busy you happened to be, your door was always open and you always took the time to answer my questions.

Then, I would like to thank Diakonhjemmet Hospital and Professor Helge Refsum, for giving me the opportunity to finish this project at Center for Psychopharmacology despite bleeding adverse drug reactions are not SFPs core field.

Throughout the work on the five included papers, I have had the pleasure of cooperating with many knowledgeable and skilful co-authors. For Paper I and II, I was lucky enough to work with experts in pharmacovigilance at RELIS who has taught me a lot, Kirsten Myhr and Vigdis Solhaug. Paper III, IV, and V were cooperation projects with the Intensive Care Unit at Oslo University Hospital, Several clinical departments at Oslo University Hospital, and The Norwegian Knowledge Centre / RELIS, respectively. I would like to take the opportunity to thank all my co-authors who have contributed to the research presented in this thesis. Special thanks to Tone Westergren and Marianne Klemp for our time consuming and exhausting, but very instructive meta-analysis project.

Also many thanks go to friends, and previous and current colleagues for their friendship, motivation and support.

Finally, I would like to thank my family and friends. Big thanks to my beloved ones, my husband Arnt Ove, and our children Øystein and Ida for your support and patience over the years of research.
LIST OF PAPERS

I. Warfarin-associated bleeding events and concomitant use of potentially interacting medicines reported to the Norwegian spontaneous reporting system.

II. Characterisation of non-warfarin-associated bleeding events reported to the Norwegian spontaneous reporting system.

III. Mortality among head trauma patients taking preinjury antithrombotic agents: a retrospective cohort analysis from a Level 1 trauma centre.

IV. Reduced Platelet Function and role of Drugs in Acute Gastrointestinal Bleeding.

V. Corticosteroids and risk of gastrointestinal bleeding: a systematic review and meta-analysis.
   doi:10.1136/bmjopen-2013-004587.

The papers are referred to by their Roman numbers in the text.
ABBREVIATIONS

ACE inhibitor Angiotensin-converting-enzyme inhibitor
ACTH Adrenocorticotropic hormone
ADR Adverse Drug Reaction
AE Adverse (drug) event
ASA Acetylsalicylic acid
ASA-PS American Society of Anesthesiologists Physical Status classification
CI Confidence interval
COX-2 Cyclooxygenase 2
CYP Cytochrome P450
DDI Drug-drug interaction
EMA European Medicines Agency
FDA Food and Drug Administration
GCS Glasgow Coma Scale
GI Gastrointestinal
INR International Normalized Ratio
MedDRA Medical Dictionary for Regulatory Activities
NISS New Injury Severity Score
NOAC novel oral anticoagulant
NSAID Non-steroidal anti-inflammatory drug
OUH-U Oslo University Hospital Ulleval
PD Pharmacodynamic
PK Pharmacokinetic
RCT Randomized controlled trial
RELIS Regionale legemiddelinformasjonscentre
SSRI Selective serotonin reuptake inhibitors
T-RTS Triage Revised Trauma Score
WHO World Health Organization
VKORC1 Vitamin K epoxide reductase 1
SUMMARY

In Norway, antithrombotic drugs have been the drugs most often reported to the Norwegian spontaneous reporting system with fatal outcome for a number of years. Antithrombotic drugs, such as warfarin and platelet inhibitors, are effective in thrombosis prevention, but are also frequently implicated in drug-induced bleeding. Drug-induced bleeding is among the most serious adverse drug reactions and contributes to a substantial morbidity, mortality, health cost and cost to society. Despite the substantial knowledge about risk factors of drug-induced bleeding, there is still a gap in knowledge with regard to risk factors and outcome among patient subgroups. Little is known about use of interacting drugs at the time of bleeding, or how physicians assess drug interactions in cases from the spontaneous reporting system. The list of drug interactions and drugs associated with bleeding is long and increasing, but for some commonly used older drugs controversy still exists regarding bleeding risk.

In this study we used different pharmacoepidemiological study designs to explore the association between the use of antithrombotic drugs and drug-induced bleeding. First, we aimed to explore reporting patterns of bleeding adverse drug reactions reported to the Norwegian spontaneous reporting system, the extent of potential drug interactions, and concordance in assessment between reporters and evaluators. Next, we used a hospital-based trauma registry to investigate whether use of pre-injury antithrombotic drugs affected mortality after head trauma. Then, in a study with case-control design, we explored whether the use of antithrombotic drugs affected platelet activity among patients admitted to hospital with gastrointestinal bleeding. Last, we investigated the association between the use of corticosteroids and risk of gastrointestinal bleeding in a systematic review and meta-analysis.
1 INTRODUCTION

1.1 Background

The history of drugs is filled with unexpected adverse drug reactions and adverse drug reaction disasters and tragedies. In fact, the history of pharmacovigilance and drug regulation parallels the history of major drug reaction disasters such as the Elixir Sulfanilamide mass poisonings in the United States in the 1930s (caused by a nephrotoxic ingredient in the liquid form of sulfanilamide) and the thalidomide disaster in Europe in 1961 (where thousands of infants were born with malformation of the limbs) [1, 2]. During the later years, England experienced the TGN1412 trial disaster in 2006 (an immunomodulatory drug inducing severe inflammatory reactions in a first-in-man study) [3] and in the Nordic countries the increased incidence of narcolepsy in children after the swine flu vaccine Pandemrix in 2009 has been called a scandal [4]. The above examples were more or less unexpected adverse drugs reactions based on the evidence available on drug approval or when the drugs were marketed and resulted in many headlines in newspapers and journal articles. The majority of adverse drug reactions, however, does not catch headlines and occur from predictable or known reactions to established drugs. Often, many of these adverse effects are not recognised on drug approval, but emerge after some years of drug use. An example is the cox-2 inhibitors and the increased risk of myocardial infarction (the Vioxx case in 2004) [5].

Drugs are tested in idealistic patient populations, but are in practice used by patients from other age groups than tested, patients with several diseases who use concomitant drugs, for other conditions than approved, and even by pregnant women. Concomitant use of several drugs (often described as polypharmacy) is common, particularly among the elderly [6]. Polypharmacy may cause drug-drug interactions and drug-disease interactions that may cause adverse events. Usually, the more severe the adverse events are, the more infrequently they occur. However, rare events may occur quite often in a population if a substantial amount of patients use a particular drug or drug combination.

Antithrombotic drugs, such as anticoagulants and platelet inhibitors, are commonly used drugs due to their documented effects in prevention of cardiovascular diseases. These drugs exert their pharmacological effects by decreasing platelet aggregation or preventing coagulation, thus decreasing thrombus formation. Because of their effect on haemostasis, drug-induced bleeding is predictable and can many times be foreseen. Drug-induced bleeding is often severe and cause substantial morbidity and mortality. Since use of
antithrombotic drugs in treatment and prevention of cardiovascular diseases is common in the western world, bleeding adverse events affect a substantial number of patients.

In Norway, adverse drug events reported to the Spontaneous Reporting System (RELIS) show that antithrombotic drugs are the drugs most often reported as associated with fatal outcome [7]. In addition, drugs with main effects on other organ systems may have pleiotropic effects on blood platelets, giving rise to adverse effects such as gastrointestinal bleeding or perforation. Polypharmacy may change absorption, distribution, metabolism or elimination of drugs, giving rise to bleeding drug-drug interactions. With a population growing older combined with a more aggressive medical treatment of the elderly, the results from randomized controlled trials might not be valid for today’s medical panorama [8]. For older drugs, such as warfarin and corticosteroids, the clinical testing before the drug came to the market was not so rigorous. Thus, smaller adverse effects or adverse effects in subpopulations may not have been scrutinised. Studies with a pharmacoepidemiological approach is thus necessary to explore how factors such as age, sex, health conditions, and concomitant medications might affect the risk of bleeding and also the outcome after bleeding adverse events.

1.2 Definition of the field

1.2.1 Pharmacoepidemiology

Pharmacoepidemiology is the study of the use of and the effects of medicinal products in large numbers of people. It applies the methods of epidemiology to the content area of clinical pharmacology [1]. The field of pharmacoepidemiology, which can be viewed as a bridging science spanning pharmacology and epidemiology, has primarily concerned itself with the study of adverse drug effects. Additionally it includes the subfields such as drug utilisation and drug use patterns in populations, pharmacovigilance, health economics and risk management, and comparative effectiveness research.

1.2.2 Pharmacovigilance

According to the World Health Organization (WHO) definition, pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems [9]. The aims are to improve patient safety and public safety in relation to drug use, to contribute to the assessment of benefit, harm, effectiveness and risk of drugs, to encourage the safe, rational and more effective use of drugs, and to promote understanding of pharmacovigilance. Pharmacovigilance can be considered as a subgroup of pharmacoepidemiology, and
includes work done by the spontaneous reporting systems. Also, pharmacovigilance is an arm of patient care.

### 1.2.3 Basics of Pharmacology

**Pharmacokinetics (PK)** characterizes what the body does to the drug and refers to the study of the absorption, distribution, metabolism, and elimination of the drug in the body (abbreviated ADME) [10]. Absorption describes the process of drug transfer from its site of administration until it reaches the systemic circulation, i.e., how much of the administered drug that reaches the blood stream. Distribution refers to how the drug is transferred into different body compartments (e.g., muscle, organs, bones, fat, and central nervous system), i.e., how much drug that is unbound in the blood stream. Metabolism is how the drug is chemically changed, mainly in the liver by the cytochrome P450 enzymes (CYP-enzymes), to active or more water soluble inactive metabolites before being excreted by the body. Elimination describes how the drug is removed from the body, usually through the kidneys or faeces, i.e., the amount of blood from which all unbound drug is removed per unit time.

**Pharmacodynamics (PD)** characterizes what the drug does to the body, both intended beneficial effects and harms. It is the study of the biochemical and physiological effects of drugs in the body, the mechanisms of drug action and the relationship between drug concentration and effect [10].

**Pharmacogenetics (PG)** is the study of how genetic differences in for example drug-metabolizing enzymes can affect the body’s response to drugs. The most widely used anticoagulant, warfarin, is metabolized by the enzyme CYP2C9 and exert its effect by inhibiting vitamin K epoxide reductase 1 (VKORC1), an enzyme that recycles vitamin K1. Gene variants in CYP2C9 and VKORC1 may explain a large part of the dose variation between patients.

**Drug interactions** occur if the effect of a particular drug is altered when it is taken together (concomitantly) with another drug, herb, supplement, or with food. Drug interactions are often described as drug-drug interactions, drug-food interactions, or drug-herb interactions and may have pharmacokinetic- or pharmacodynamic consequences or both. A pharmacokinetic interaction alters the concentration of the drug in blood and may easily be detected by therapeutic drug monitoring or by measuring INR (for warfarin). Pharmacodynamic interactions may occur if other drugs have additive or antagonistic effects on the same enzymes / receptors or by other changes at the cellular level, but will not change the serum concentration of the drug.
1.2.4 Definitions of drug-induced bleeding

Several drugs can lead to bleeding, which is usually an adverse effect of the drugs’ intended actions. The terms adverse effect, adverse event, side effect and adverse (drug) reaction are often used interchangeably to describe noxious drug effects, but according to the WHO definitions, each term has its own definition [9, 11]. An adverse (drug) reaction (ADR) is defined as a response to a medicinal product which is noxious and unintended, and which occurs at doses normally used in humans for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function. The term adverse (drug) effect is a synonym. An ADR is characterized by the suspicion of a causal relationship between the drug and the occurrence. An adverse event/adverse experience, in contrast, is defined as any untoward medical occurrence that may appear during treatment with a pharmaceutical product which not necessarily have a causal relationship with the treatment. A side effect is defined as any unintended (positive or negative) effect of a pharmaceutical product occurring at normal dosage which is related to the pharmacological properties of the drug. Harms are defined as the totality of possible adverse consequences of an intervention or therapy; they are the direct opposite of benefits [12]. In comparative effectiveness research, i.e. in systematic reviews, harm is the preferred term. In contrast, safety, which is defined as a substantive evidence of the absence of harm, is the term mostly used in randomized controlled trials (RCTs). The term drug related problem (DRP), defined as an event or a circumstance involving drug treatment that can actually or potentially interfere with the patient care, is used in pharmaceutical research. A medication error is any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the health care professional, patient, or consumer. It is used in the field of healthcare quality and safety. The preferred terms used in pharmacovigilance are adverse (drug) reactions and adverse (drug) effects. In 2012, the European Medicines Agency (EMA) and the Norwegian Medicines Agency adopted a new definition of ADR which includes the use of a medicinal product within the terms of the marketing authorisation, outside the terms of the marketing authorisation, including overdose, off-label use, misuse, abuse and medication errors and occupational exposure [13]. The WHO definition of ADRs is used in this thesis.

1.2.5 Bleeding disorders

Bleeding may be caused by a variety of conditions, including bleeding diathesis (an unusual susceptibility to bleeding mostly due to hypocoagulability), specific dispositions such as hypertension or Helicobacter pylori infection, or external factors such as traumas. Bleeding diathesis may be inherited, such as haemophilia and von Willebrand disease, or may be
acquired. Acquired causes of bleeding diathesis include, but are not restricted to, anticoagulation with warfarin, use of other antithrombotic drugs, or drug interactions, vitamin K deficiency, liver failure, autoimmune causes, following severe illness with septic shock, and malignancies like leukaemia [14].

1.2.6 Drugs associated with bleeding

Drugs obviously related to bleeding are those with effect on coagulation, thrombus formation, or fibrinolysis such as anticoagulants, platelet inhibitors, and fibrinolytics, respectively. The list of drugs associated with bleeding and drugs suggested to increase bleeding risk, however, is much longer and includes drugs with the main effect on other organs and effect on haemostasis as a pleiotropic effect. Drugs with well-known pleotrophic effect on haemostasis include non-steroid anti-inflammatory drugs (NSAIDs) and (selective) serotonin reuptake inhibitors (SSRIs) [15, 16]. Other drugs that may influence on the haemostasis (as documented in small clinical studies or in in vitro studies) include paracetamol [17], angiotensin receptor blockers [18], calcium antagonists [19], statins [20, 21], non-pharmaceutical products such as herbal products and nutriceuticals [22, 23], and alcohol [24]. In other studies, some of these associations have been refuted [15, 25, 26]. It has been a long-standing debate whether corticosteroid therapy leads to peptic ulcer or gastrointestinal bleeding [26-30]. Corticosteroids have been on the market since the late 1940s, and illustrate how difficult it may be to reach definitive conclusions regarding adverse drug effects. In addition, drugs, food and herbal products may interact with drugs with a well-known bleeding risk, giving rise to a further increase in bleeding risk [31-33]. Bleeding may occur from all parts of the body, but warfarin and fibrinolytic drugs seem to give more intracranial haemorrhages than the other drugs.

The Norwegian Prescription Database includes data about dispensed drugs in Norway. In 2004 warfarin was prescribed to 73 000 persons in Norway. Of those, 22 000 were 80 years and older. Acetylsalicylic acid, in prevention of cardiovascular disease, was prescribed to almost 300 000 persons. Of those, 74 000 were 80 years and older. Drugs dispensed to individuals during a hospital stay or in nursing homes are not recorded in The Norwegian Prescription Database. This will underestimate the total use of antithrombotic drugs in the population, especially among the elderly people.

1.2.7 Incidence of adverse drug reactions

Adverse drug reactions are considered to contribute to a substantial morbidity, mortality, health cost and costs to society. Adverse drug reactions as reason for hospital admissions are quite frequent. In a British study, 1225 of 18 820 admissions to hospital (6.5 %) was
related to an ADR, accounting for 4% of the hospital bed capacity [34]. Similar findings have been reported from Italy (3.4% of all admissions in the elderly population) and the Netherlands (3.2% of hospitalizations were drug related in the ≥ 80 years group) [35, 36]. Drug related deaths have been explored in several studies. In a Norwegian study of fatal adverse drug events in a department of internal medicine, 18% of the deaths were classified as being directly or indirectly associated with one or more drugs (which equals 9.5 deaths per 1000 hospitalized patients) [37]. In a corresponding meta-analysis, fatal ADRs were suggested to be among the six leading causes of in-hospital deaths in the United States, with an incidence of 6.7% serious ADRs and 0.32% fatal ADRs among hospitalized patients (including ADRs occurring during hospitalization and ADRs causing admission to hospital) [38]. In a Swedish study of deceased study subjects, 3.1% were suspected to have died from fatal ADRs [39]. In contrast, the extent of adverse drug events in ambulatory care not sufficiently severe to cause hospitalisations remains more unclear. In a recent Swedish study, however, the economic cost of outpatient care accounted for 45% of all direct costs caused by the adverse drug events [40].

The total clinical and economic burden of drug-induced bleeding, however, is not easy to estimate according to differences in materials, methods, and the criteria for classification used to detect the unwanted drug effects. In addition, temporal trends in treatment with antithrombotic drugs may affect the incidence of adverse drug reactions and thus the burden to the society. This has been documented for warfarin, which has become increasingly common among elderly people and consequently causes an increase in the incidence of intracranial bleeding [41]. Bleeding adverse drug reactions may occur in all parts of the body. However, the severity and the mortality vary considerably across different bleeding localizations. Intracranial bleedings are the most severe and is associated with substantial mortality [42], with an estimated Norwegian incidence rate in 2010 of 40 per 100 000 persons per year and a mortality rate of 20 per 100 000 persons per year, i.e. a 50% mortality [43]. Gastrointestinal bleedings (bleeding peptic ulcer) may be severe, and is also associated with substantial mortality. In Sweden, hospitalisations for bleeding peptic ulcer was 35 per 100 000 inhabitants per year in 2005 and the overall mortality was 6% [44].

The risk of major bleeding in patients treated with warfarin is related to degree of anticoagulation as well as the presence in the patient of pre-existing risk factors for bleeding [45]. From randomized controlled trials, bleeding rates in the range of 1 to 3 percent per person year have been reported [45, 46]. RCTs are performed in idealistic patient populations (i.e. exclusion of older patients, patients with co-morbidity, and patients who use specific drugs). Thus, the results may have low generalizability (external validity) to other patients than included in the RCTs [47] and the bleeding frequencies that occurs in clinical
practice may be underestimated. In observational studies annual bleeding rates up to 10% has been reported in inception-cohort studies and among old, frail patients [48, 49]. Bleeding rates of 15 per 100 patient years have been reported when minor bleeds are included [50].

Platelet count and platelet function are of vital importance for normal haemostasis. NSAIDs are associated with an increased risk of gastrointestinal bleeding, the risk increase, however, varies considerably between studies. An almost 80% increased occurrence of gastrointestinal bleeding with NSAIDs use was reported in a case control study from the British General Practitioners Research Database (rate ratio 1.78, 95% CI 1.61-1.97) [33], compared to an almost four times increased occurrence of gastrointestinal bleeding/ perforation in a systematic review (pooled relative risk 3.8, 95% CI 3.6-4.1) [51]. Some variation in risk between individual NSAIDs has been shown, though the differences are substantially less pronounced when comparable daily doses are considered [51]. Corresponding results have been shown for acetylsalicylic acid, clopidogrel, and dipyridamole with 40 – 80% increased risk of gastrointestinal bleeding [33, 52]. SSRIs have been reported to increase the occurrence of upper gastrointestinal bleeding. Adjusted odds ratios of 1.6 (95% CI 1.2-2.1) and 2.9 (95% CI 1.5-5.6) for SSRIs and SNRIs, respectively, were reported in a nested case control study [53].

1.2.8 Mechanisms of antithrombotic drugs

Anticoagulants such as vitamin K antagonists (warfarin) and the new per oral anticoagulants (apixaban, rivaroxaban, and dabigatran) affect the coagulation system. Warfarin interfere with the formation of several vitamin K-dependent clotting factors (factors II, VII, IX, and X) and anti-coagulant proteins (protein C, -S, and –Z). In contrast, the new per oral anticoagulants inhibit either factor Xa (apixaban and rivaroxaban) or thrombin (dabigatran). Heparins prevent clot formation and extension of existing clots within the blood by binding to antithrombin III, which inactivates thrombin. Heparin does not break down already formed blood clots in contrast to fibrinolytic drugs (tissue plasminogen activator, streptokinase), which dissolve blood clots by activating plasminogen to plasmin.

Platelet inhibitors inhibit platelet function by binding to different receptors on the platelet surface. Acetylsalicylic acid (ASA) irreversibly acetylates cyclooxygenase (COX) – and thereby inhibits thromboxane A2-mediated platelet aggregation as well as prostaglandin E2-mediated gastro protection. NSAIDs are non-selective and reversible inhibitors of both COX-1 and COX-2; however, the inhibition of COX-1 varies between the agents. Clopidogrel irreversibly inhibits the P2Y12 receptor (ADP receptor) on the platelet cell membrane.
1.2.9 Measurement of anticoagulant and antiplatelet activity

Several blood tests exist to assess clotting tendency, such as APTT (partial thromboplastin time), thrombin time, anti-factor Xa activity, and INR (International normalized ratio). INR is a measure of the extrinsic pathway (initiated when tissue damage) of coagulation, and is used in the follow-up of patients using warfarin. The target range for INR during warfarin use is 2 to 3 [54]. INR can easily be measured in most laboratories and even by patients themselves at home. In contrast, platelet function is not as easily measured due to the platelet reactivity. As a consequence, platelet function, except from platelet count, is not measured in routine clinical practice. Platelet function tests, such as whole-blood aggregometry and flow cytometry among others are, however, used for research purposes [55]. Flow cytometry is used to measure platelet activation, while aggregometry measures time to platelet aggregation.
1.3 Risk factors for drug-induced bleeding

1.3.1 Age

The use of anticoagulant therapy in elderly patients is a dilemma because of a higher bleeding risk and a higher mortality following major bleeding in this patient group [56]. The increased risk has been shown in both pooled data from RCTs [57] and from observational studies [48, 56]. In an observational study of patients treated with oral anticoagulation because of mechanical heart valves, atrial fibrillation or after myocardial infarction, the incidence of major bleeding increased sharply with advanced age; from 1.5 per 100 patient-years for patients younger than 60 years to 4.2 per 100 patient-years for patients older than 80 years [56]. In a corresponding study of elderly patients during the first year of warfarin therapy, patients ≥80 years of age experienced 13.1 major bleedings per 100 patient-years, compared to 4.8 major bleedings per 100 patient-years for those < 80 years [48]. In a study based on pooled data from >30 000 patients with acute coronary syndromes, patients with major bleeding were older and had a 5-fold higher incidence of death during the first 30 days.

Together with increased bleeding risk, stroke rates rise substantially as patients get older (the relative risk increases by a factor of 1.5 per decade), and most strokes due to atrial fibrillation occur in patients over the age of 75 years. Numerous studies have documented an underutilization of oral anticoagulant therapy among older patients, particularly those who are at highest risk of stroke [58]. The underutilization of oral anticoagulants occurs due to fear of bleeding, and despite the proven benefit of anticoagulant therapy [59].

1.3.2 Start of therapy

Several studies have shown that the initial phase of anticoagulant therapy conveys the highest risk of bleeding. Using an inception cohort of patients on long term outpatient warfarin therapy (median duration of therapy was seven months (range <1 month – 97 months)), Landefeld found that the monthly risk of major bleeding decreased sharply from 3% during the first month of therapy to 0.3% per month after the first year [60]. In a study by Hylek et al, where elderly patients were enrolled on the first day of warfarin treatment and followed through the first year, the first 90 days of warfarin treatment was associated with a 3-fold increased risk of major haemorrhage (defined as fatal, hospitalisation with blood transfusion, or involvement of a critical site) [48]. Forty-two per cent of the major haemorrhages occurred within 30 days of warfarin initiation. In a corresponding multi-centre cohort study by Palareti et al, patients were followed from the start of their oral anticoagulation (warfarin or acenocoumarol, and mean follow-up was 267 days) [61]. The relative risk of any haemorrhagic event (fatal, major or minor) was 1.8 times higher during the
first 90 days of treatment, with an occurrence of 11 bleeds per 100 patient years during the first 90 days. In studies based on data from spontaneous reporting systems, the results have been conflicting. In a previous Norwegian study, most reported haemorrhages (63 %) occurred during the first month of therapy, and one third occurred during the first five days of treatment [62]. In a Swedish study, in contrast, most reported haemorrhages (64 %) occurred after more than 12 months of treatment [63]. It has previously been a common practice of initiating warfarin therapy with a loading dose [64]. This may have led to a significant over-anticoagulation, particularly in elderly. As a result, a Norwegian guide to safer anticoagulation was published in 2005 [65]. It is not known if more cautious initiation of warfarin therapy has resulted in fewer very early bleeding ADRs or altered reporting patterns.

1.3.3 Drug interactions and assessment of interactions

Drug interactions occur frequently and are an important cause of adverse drug reactions. The interactions occur mostly unintended and represent a widely under-recognized source of medical errors. Drug interactions may be pharmacokinetic, pharmacodynamic, or both. The pharmacodynamic interactions that are apparent from the main pharmacological action of the drugs, i.e. additive or antagonistic effects, are often intuitive. The pharmacokinetic interactions are often less intuitive due to metabolism via different CYP-enzymes. In order to guide health care personnel to recognize potential drug interactions, several drug information compendia exist such as the Norwegian National Formulary (Legemiddelhåndboken), the British National Formulary (BNF) and the American Martindale [66-68], in addition to automated drug alerts in the electronic prescription systems. The different drug alert systems and drug information compendia, however, show substantial differences in included interactions [69]. The lack of agreement is caused by differences in methodologies (including / excluding herbs or food, listing of individual drugs, combination products, or drug classes), differences in severity classifications, and the lack or sparseness of documentation on the underlying evidence base. Pharmacodynamic interactions that are apparent from the main pharmacological action of the drugs are often undersupplied in the drug information compendia. All these contribute to explain the different occurrences of potentially interacting drugs between studies. The science of drug interactions derives its knowledge from case reports and experiments in healthy volunteers. There is a paucity of controlled experiments exploring the consequences of drug interactions in real-world patients, primarily because patients with concomitant diseases are often excluded from RCTs. In addition, observational studies require large databases and linkage of prescription records with laboratory data or clinical outcomes.
From prescription database studies it has been shown that up to 80% of the anticoagulant users have been prescribed potentially interacting drugs [70]. In hospital based studies with warfarin the percentages [71] are lower (40-60%), but still high [72-74]. In a study from the Norwegian Prescription Database, focusing on co-prescription of warfarin with analgesics and NSAIDs/COX-2 inhibitors, at least 25% was prescribed a concomitant drug [75]. Warfarin is known for its many interactions with drugs, food, and herbal products [76]. The drug is old and has been used for more than 60 years, however, new interactions, such as interactions with vaccines [77], cough remedies [78], and analgesics [79], continue to emerge. Due to a narrow therapeutic range with warfarin, drug interactions may cause severe consequences such as intracranial or gastrointestinal bleeding. All types of drugs, however, may be implied in drug interactions, but the clinical consequences are highly variable and most often clinically insignificant [80]. This is because the majority of potential interactions only cause minor effects or no effects at all, making a high signal to noise ratio.

Several studies have been performed to assess the bleeding risk associated with drug-drug interactions (DDI’s). Concurrent use of warfarin and potentially interacting drugs has been associated with a 2 to 7-fold increased risk of major bleeding [33, 72, 81]. Similar increases in risk of serious upper gastrointestinal bleeding with the use of combined antithrombotic therapy (acetylsalicylic acid, clopidogrel, dipyridamole, or warfarin) were found in a Danish case-control study [52]. NSAIDs are associated with gastrointestinal toxicity, and are among the major cause of upper gastrointestinal bleeding in adults. Use of NSAIDs with concurrent drugs such as acetylsalicylic acid, corticosteroids, SSRIs, or oral anticoagulants has shown both additive and synergistic effects [82, 83]. Loke et al found that combined use of SSRIs and NSAIDs was associated with increased incidence of gastrointestinal bleeding (odds ratio 6.33, 95% CI 3.40-11.82) compared with use of either SSRIs (odds ratio 2.36, 95% CI 1.44-3.85) or NSAIDs alone (odds ratio 3.16, 95% CI 2.40-4.16) [83].

Studies from the spontaneous reporting system on bleeding adverse drug interactions are more limited, but include data from the French and the Italian spontaneous reporting systems. Clinard et al reported ADRs associated with use of several systemic NSAIDs in a case-control design [84]. The French database comprised almost 55,000 spontaneous reports between 1995 and 2000 and 194 cases of gastrointestinal bleeding. The use of NSAIDs increased the occurrence of gastrointestinal bleeding, odds ratio 7.3 (95% CI 4.9-10.9) with use of one NSAID and odds ratio 10.7 (95% CI 2.9-40.2) with two or more NSAIDs. The ADR cases were assessed and recorded by the medical team at the pharmacovigilance centre. In contrast, there was no focus on the reporters’ assessments of potential drug interactions in each report, or description of concomitant use of other drugs with potential to affect haemostasis. Leone et al identified ADRs associated with drug-drug interactions
reported in Italy between 1990 and 2007 [85]. Nearly 18 000 reports containing at least two drugs were included in the analysis. In 5 300 reports (30.2 %) there were at least one potential DDI, and in 1 200 reports (6.5 %) an ADR was associated with the DDI. Anticoagulant and antiplatelet drugs had highest mortality. In 20.3 % of the reports both interacting drugs were recognized as suspect by the reporter, but the percentages varied considerably between drug-drug pairs. DDI’s between antithrombotic drugs were recognized as suspect by the reporter in 65% of the case reports. Bleeding caused by drug-drug interactions is well known and documented. Leone et al. comments on the poor awareness among reporters on bleeding DDI’s, as only one drug was indicated by the reporter as suspect in more than one-third of the cases.

Spontaneous reports from known ADRs can provide insight into inappropriate co-prescribing of medications and potentially drug-drug interactions [86]. Most studies from the spontaneous reporting system, however, focus on the offending drug. Others focus on the outcome, such as fatal ADRs [63, 87, 88], or describe trends in the reporting [89, 90]. Little attention has been given to the potential value of the detailed content of the ADR reports. Additionally, there has been little focus on search in the database based on adverse events (MedDRA terms) such as bleeding events, or the use of spontaneous reporting databases for describing the reporters assessments of potential DDI’s.

1.3.4 Intensity of anticoagulation

The intensity of anticoagulation with warfarin has to be strictly controlled, as warfarin has a narrow therapeutic range. Time spent with an INR above the therapeutic range increases the risk of bleeding, while time spent below the therapeutic range increases the risk of thrombosis. Clinical trials during the past 25 years have generated this knowledge, and have revolutionized the antithrombotic treatment. Previously, a fixed low-dose warfarin was suggested as effective prophylaxis in stroke prevention avoiding the excess bleeding risk associated with adjusted-dose warfarin. A low-dose warfarin, however, was shown to be insufficient for stroke prevention and conveyed similar bleeding rates as warfarin in adjusted-dose regimes [91]. For most indications, including venous thromboembolism, an INR value between 2.0 and 3.0 is targeted. Some patients, i.e. patients with mechanical heart valves, need a target INR between 2.5 and 3.5. This reduces the risk of ischemic stroke by about 67 % compared to placebo. It has been shown that the risk of ischemic stroke and bleeding rises steeply at an INR below 2.0 [54] and above 3.5 - 5.0, respectively [45, 92]. A decrease in INR to 1.7 doubles the risk of thrombosis compared to an INR of 2.0 [54]. Likewise, an INR higher than 4.5 conveys a 12 times increase in bleeding risk compared to an INR in therapeutic range [45]. Several factors may affect the degree of anticoagulation. Warfarin maintenance
dose is inversely related to age and is strongly associated with gender [93]. Among ambulatory patients with an INR target between 2.0 and 3.0, the median weekly dose range from 45 mg for men younger than 50 years of age to 22 mg for women 80 years or older. Since warfarin dose requirements decrease greatly with age, the commonly employed empiric starting doses of 5 – 10 mg/d will lead to over-anticoagulation for the majority of patients in the geriatric age group. Besides age, pharmacogenetic variability may explain a large part of the dose variation between patients. Carriers of allelic variants in CYP2C9 and VKORC1 have been shown to experience hyper-responsiveness to small doses of warfarin and a higher bleeding rate [45].

1.3.5 Traumas

The use of antithrombotic agents is prevalent among the aging population and thus among trauma patients. There is, however, limited documentation whether pre-injury use of warfarin or platelet inhibitors affects outcome after blunt head trauma. Studies investigating trauma without focusing on head trauma in particular have shown conflicting results; older studies has failed to document increased mortality in patients using warfarin [94, 95], while increased mortality has been found in a more recent, and very large trauma database study [96]. Studies focusing on head trauma have also shown conflicting results [96-102], as no increased mortality risk was seen among subgroups of patients 65 years and older with intracranial haemorrhage or among a subset of patients with head injury [96, 102]. In some studies, the therapeutic anticoagulation (INR), not warfarin use itself, has been shown to be important [98, 103-105], indicating that anticoagulants may play a mechanistic role in the adverse outcomes and not serve as markers for co-morbidity that leads to worse outcome. Whether the use of platelet inhibitors is associated with increased risk of intracranial bleeding or affects mortality after head trauma is even less well studied. In some studies platelet inhibitors are associated with increased risk of mortality, and with even higher mortality compared to warfarin (20-47%) [106, 107], while other studies show no increased risk [108, 109].

The studies on outcome after head trauma are retrospective and of observational design. Additionally, they vary with respect to which types of trauma mechanisms that are included (all traumas, falls, fall from standing), which body parts that are affected (all body vs head traumas), possible confounding factors like age (all ages, 18/55/65 years and older) and trauma severity (minor injuries, Glasgow Coma Scale 14-15, haemorrhagic brain injury), and outcome measurements (mortality [until discharge, 30-day], intracranial haemorrhage, length of stay, severity of brain injury, Glasgow Outcome Scale), making comparisons between studies difficult. More recently, however, two meta-analyses were performed in order to
determine the effect of preinjury antithrombotic drugs on mortality in patients with blunt head trauma [110, 111]. Preinjury warfarin use was associated with a two times increased risk of mortality following blunt head trauma (odds ratio 2.01, 95% CI 1.63-2.47). Use of preinjury clopidogrel (odds ratio 1.55, 95% CI 0.32-7.54) and acetylsalicylic acid (odds ratio 2.44, 95% CI 0.64-9.31) both showed an insignificant increased risk of mortality. The meta-analyses, however, included case control studies, cohort studies, and nested case control studies and adjustment for differences in trauma severity, physiological derangement (GCS), and age was not performed in the meta-analysis. The meta-analyses may thus include confounding factors that are not adjusted for.

1.3.6 Corticosteroids and bleeding

An association between corticosteroid use and peptic ulcer, gastrointestinal bleeding or perforation has been a source of debate since the 1950s [26, 27, 30]. The first corticosteroids (cortisone) and adrenocorticotropic hormone (ACTH, which stimulates biosynthesis of corticosteroids) were introduced to the American market in the late 1940s. A couple of years later case reports describing activation or aggravation of peptic ulcers and development of gastrointestinal perforation during ACTH therapy emerged in the medical literature [112, 113]. Precise descriptions followed on how ACTH or steroids may mask symptoms of infection and abdominal pain. Documentation followed on how ACTH depresses all the elements of granulation tissue in animals. Since the healing of a peptic ulcer is dependent on healthy granulation tissue at the base of the ulcer, it was considered conceivable that the action of the hormone could depress ulcer healing in humans in a similar manner, predisposing to gastrointestinal bleeding or perforation. The knowledge of corticosteroids as ulcerogenic, however, was based on case reports and case series. Later, several researchers have tried to prove or disprove this association in trials based on patient populations.

Three meta-analyses published in 1976, 1983, and 1994, have been conducted to clarify whether corticosteroids increase the risk of gastrointestinal bleeding or perforation [26, 27, 30]. The first studies included several ambiguities in the randomization procedures, double-blindedness, selection criteria, and presentation of complications. In the first meta-analysis, Conn and Blitzer reported an ulcer rate in double-blind studies of 1.4 % in the steroid-treated patients compared to 1.0 % in the controls, and thus concluded with a non-association between corticosteroid therapy and peptic ulcer because of the statistically insignificant difference [30]. Messer and colleagues came to the opposite conclusion in a meta-analysis published in 1983 [27]. They found ulcer rates of 1.8 vs 0.8 % (P<0.001) for all trials and 2.6 vs 1.5 % (P=0.04) when only double-blind RTCs were included [27]. Conn and Poynard published an updated meta-analysis in 1994, this time with inclusion of double-blind RCTs
only [26]. The literature search, however, was performed between 1950 and 1982, and was in fact a reanalysis of Messer’s data. The authors stated that a rapid survey indicated that there had been relatively few new double-blind RCTs after 1982. There was a non-significant increase in ulcers, haemorrhage, and perforation, and the authors concluded with a non-association between corticosteroids and peptic ulcer disease.

Results from case control studies and population-based cohort studies are likewise conflicting. Hernandez-Diaz and Garcia Rodriguez used a case-control design in a study based on the General Practice Research Database in the United Kingdom [114]. The risk of upper gastrointestinal complications was 1.8 (95% CI 1.3-2.4) times higher for users of oral corticosteroids than for nonusers. A nested case control study of computerised Medicaid files later gave a relative risk for hospitalisation for peptic ulcer of 2.0 (95% CI 1.3-3.1) with corticosteroid use [115]. The risk, however, was not increased without concomitant use of NSAIDs (1.1, 95% CI 0.5-2.1). In Denmark several population-based cohort studies have been performed [29, 116, 117]. In the studies, use of corticosteroids has been associated with a 2.9 fold increase in hospitalisation for gastrointestinal bleeding and a twofold increase in 30-day mortality among patients hospitalised with perforated peptic ulcer. In contrast, current use of corticosteroids without use of other ulcer-related drugs conveyed an insignificant increased risk of mortality (1.3, 95% CI 0.81-2.08) following bleeding peptic ulcer. A problem with these studies is how much the underlying disease accounts for the increased bleeding risk.

In 2007 Fardet and colleagues did an attempt to provide an overview of the available data regarding the frequency of several adverse events observed in adults during systemic corticosteroid therapy [118]. They included RCTs and meta-analyses, but due to the search methodology, i.e. inclusion of the term “adverse event” in the search strategy, only 244 papers were retrieved, and only 19 were included in their review. Regarding peptic ulcer, their conclusion was based on the results from Conn and Messer. Newer Cochrane meta-analyses have addressed the question in selective patient populations. These analyses show a trend [119, 120] or a statistically significant increase [121] in the risk of experiencing GI bleeding. Thus clinical recommendations, databases, and product monographs for corticosteroid associated peptic ulcer disease or gastrointestinal bleeding still refer to the meta-analyses by Conn and Messer. Peptic ulcer disease and gastrointestinal bleeding may or may not be described as possible adverse effects of corticosteroids, but seldom is the paucity of newer documentation debated.
2 AIMS

Despite the substantial knowledge about risk factors of drug-induced bleeding and bleeding outcome, there is still a gap in knowledge about risk factors among patient subgroups, for rare events, and even for commonly used drugs. The overall purpose of this thesis was to explore drug-induced bleeding. The specific objectives were as follows:

1. To describe reporting patterns of bleeding adverse drug reactions reported to the Norwegian spontaneous reporting system (Paper I and II)
2. To examine the extent of potential drug interactions in the spontaneous reports and concordance in assessments between reporters and evaluators (Paper I and II)
3. To assess whether use of pre-injury antithrombotic drugs affect mortality after head trauma, using a hospital based trauma database (Paper III)
4. To measure platelet activity in patients admitted to hospital with acute gastrointestinal bleeding (Paper IV)
5. To use systematic review / meta-analysis of RCTs to assess whether corticosteroid use is associated with increased risk of gastrointestinal bleeding or perforation (Paper V)
3 MATERIALS AND METHODS

3.1 Patient selection and study design

Paper I and II are retrospective cohort analyses based on data from the Spontaneous reporting system database at the Norwegian Medicines Agency. The database captures case reports received by the regional pharmacovigilance centres and case reports collected by the drug companies. A database search was performed from 1 January 2003 to 31 December 2005 for bleeding events based on MedDRA terms (a standardised medical terminology dictionary). All reports with bleeding events from any part of the body and among all age groups were included. Reports with and without use of vitamin K antagonists were analysed separately in Paper I and Paper II, respectively.

Paper III is a retrospective cohort analysis based on data from the hospital-based trauma registry at Oslo University Hospital Ulleval (OUH-U). The hospital is the major trauma hospital for 550,000 citizens from Oslo and surrounding areas and the trauma referral centre for 2.5 million people from South-Eastern Norway. Patients 55 years or older with documented head trauma admitted to OUH-U between 1 January 2004 and 31 December 2006 and registered in the trauma registry were included.

Paper IV is a retrospective case-control study based on patients (n=35) recruited consecutively on admission to OUH-U. Inclusion criteria were clinical presentation of acute hematemesis and/or melena. Treated controls (n=24) without bleeding were randomly selected patients with coronary heart disease from the ASCET (Aspirin non-responsiveness and Clopidogrel Endpoint Trial) population. Untreated healthy controls (n=27) were volunteers recruited from laboratory and hospital personnel. The study participants were recruited between 2006 and 2008.

Paper V is a systematic review and meta-analysis of randomized, double-blind, controlled trials comparing a corticosteroid to placebo for any medical condition or in healthy participants. The databases MEDLINE and EMBASE were searched between 1983 (since date of the latest review by Conn and Poynard) and 30 June 2011. An additional search was performed in the Cochrane Database of Systematic Reviews for corticosteroids and specific text words. Use of concomitant drugs was allowed and no age groups were excluded.

3.2 Data collection

Paper I and II are based on data from the Spontaneous reporting system database. From each retrieved report the age, sex, and the outcome (mortality) of the patient were recorded.
The bleeding localisation, together with all the drugs documented in the reports, the reporters` assessments of a drug as suspect or interacting to cause the bleeding, the duration and the indication for drug use, and INR values were recorded. The bleeding localisations were categorised according to suspected severity; cerebral, gastrointestinal or from other localisations. The Norwegian National Formulary (Legemiddelhåndboken), the British National Formulary (BNF) and Martindale were searched for drugs that may affect haemostasis and drugs that may potentially interact and increase the effect of warfarin.

From the hospital-based trauma registry (Paper III) data on trauma mechanisms, trauma severity (anatomical and physiological grading systems), and pre-injury co-morbidity were retrieved, together with individual patient data (age, sex and outcome). Pre-injury use of warfarin or platelet inhibitors and degree of anticoagulation was determined by a review of electronic medical records. The grading systems for trauma severity (NISS and T-RTS) and pre-injury co-morbidity (ASA-PS) have been shown to be predictors of outcome after trauma [122].

In patients with acute GI bleeding (Paper IV), blood samples were taken on admission to hospital, together with routinely taken blood samples before commitment to treatment. In treated controls with coronary heart disease, blood was drawn in fasting condition before intake of morning medication. In healthy controls, the blood samples were taken in the morning (non-fasting). Platelet function measurement (impedance aggregometry and flow cytometry) was performed within three hours after blood sampling.

For the meta-analysis (Paper V), titles, abstracts and full-text articles of records identified in the literature search were evaluated and reviewed for inclusion by at least two of the authors. Disagreements were resolved by consensus among the three authors. Articles with documentation of gastrointestinal adverse effects or with assessment of adverse event monitoring described in the methods section were included. For the outcome measure, gastrointestinal bleeding or perforation, the investigators` diagnoses were accepted as valid without requiring specific criteria of methods. Study characteristics and demographics as stated in the articles were recorded. Methodological quality, risk of bias and severity of disease in included articles were assessed by the authors.

### 3.3 Statistics

The statistical software used was the Statistical Package for the Social Sciences (IBM Corp. IBM SPSS Statistics for Windows, Versions 16.0 – 20) and Review Manager (RevMan) [Computer program], Version 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration.
The descriptive statistics are expressed as number and frequencies (%) for categorical variables, and mean ± standard deviation and median and range/interquartile range for normally and non-normally distributed continuous variables, respectively. The Pearson chi-square test and the Kruskal-Wallis test were used for testing categorical variables. The Mann-Whitney test and t-test were used for testing continuous variables.

A multivariate logistic regression model was used to assess independent predictors of mortality (Paper II and III). In patients with ongoing GI bleeding (Paper IV) multiple linear regression analyses were used to study the relationships between aggregation and P-selectin/CD63 expression and different variables.

The meta-analytic calculations (Paper V) were made using the Mantel-Haenszel method with the random effects model.

3.4 Legal and ethical aspects

The Regional Ethics Committee approved the studies based on data from the Spontaneous reporting system database, the Trauma Registry, and the study on ongoing GI-bleeding. The study based on data from the Spontaneous reporting system database and the Trauma Registry was approved by the local patient ombudsman. Written informed consent was obtained from all subjects in the ongoing GI-bleeding study before blood samples were taken.
4 SUMMARY OF RESULTS

Paper I

Case reports on warfarin-associated bleeding events reported during 2003-05 were retrieved from the Norwegian spontaneous reporting system database. In total, 289 case reports were included. Mean age was 75.9 years, 117 (40.5%) were female, and 139 (48.1%) had fatal bleeding. A higher proportion of the cerebral bleeding events (109/174 cases, 62.6%) than gastrointestinal bleeding events (24/69 cases, 34.8%) and other bleeding events (6/46 cases, 13.0%) were fatal. A total of 1261 drugs (median 4.0 per patient, range 1-17) was used. The evaluators identified 546 drugs including warfarin (median 2.0 drugs per patient, range 1-7) that could possibly cause bleeding alone or in combination. The reporters assessed 349 drugs (median 1.0 drug per patient, range 1-4) as suspect to contribute to bleeding. Evaluators identified 156 pharmacokinetic- and 101 pharmacodynamic drug interactions, compared with 19 pharmacokinetic- and 56 pharmacodynamic drug interactions reported as suspect by the reporters. Among the early bleeding events (37 reports with warfarin use less than 3 months), reports with warfarin without interacting drugs showed the highest INR. Among the late bleeding events (187 reports with warfarin use longer than 3 months), reports with pharmacokinetic interacting drugs had the highest INR. In conclusion, concomitant use of potentially interacting drugs was involved in the majority of the late warfarin-associated bleeding events. Reporters assessed mostly warfarin as the only contributor to bleeding. In particular, pharmacokinetically interacting drugs were not suspected as contributing to bleeding.

Paper II

Data on non-warfarin-associated bleeding events reported during 2003-05 were retrieved from the Norwegian spontaneous reporting system database. Of 327 case reports of non-warfarin-associated bleeding events, 270 reports (82.6 %) were characterised as serious and 69 (21.1 %) had a fatal outcome. 187 bleeds (57.5 %) were gastrointestinal, 57 (17.4 %) were cerebral, and 81 (24.8 %) were from other bleeding sites. The bleeding sites differed with respect to the patient’s age, drug use, diagnoses and outcomes. Of drugs associated with bleeding, NSAIDs/COX-2 inhibitors (145 reports) and ASA (128 reports) were most frequently used. In a logistic regression model, fibrinolytic drugs were the only drug associated with increased mortality. There was a 67.4 % correlation between reporters and evaluators in assessment of drugs associated with bleeding, with considerable variation in concordance between drug groups (with 100 % correlation for fibrinolytics compared to 40 % correlation for ASA/clopidogrel/dipyridamole/abciximab). In conclusion, non-warfarin-associated bleeding events were associated with substantial mortality. Old age, cerebral
bleeds, number of drugs used, and use of fibrinolytic drugs were all independently associated with increased mortality. The recognition of the bleeding risk of commonly used drugs such as ASA and heparins may be insufficient among prescribers.

Paper III

A retrospective cohort analysis was performed on the hospital based trauma registry at Oslo University Hospital Ullevål. Patients aged 55 years or older sustaining blunt head trauma during 2004-06 were included. Multivariable logistic regression analyses were used to identify independent predictors of 30-day mortality. Of the 418 patients admitted with a diagnosis of head trauma, 137 (32.8 %) used any pre-injury anticoagulant and/or platelet inhibitor (53 warfarin, 80 platelet inhibitors, and 4 both). The main trauma mechanisms were falls (257, 61.5 %), followed by motor vehicle accidents (110, 26.3 %). Seventy patients died (16.7 %); 15 (28.3 %) of the warfarin users, 12 (15.0 %) of the platelet inhibitor users, and two (50 %) with combined use of warfarin and platelet inhibitors, compared to 41 (14.6 %) of the non-users. There was a significant interaction effect between physiological status on admission (T-RTS) and warfarin. After adjusting for important covariates, warfarin use was associated with increased 30-day mortality among patients with normal physiology (T-RTS=12) (odds ratio 8.3, 95% CI 2.0-34.8), but not among patients with physiological derangement (T-RTS≤11) (odds ratio 1.2, 95% CI 0.4-3.1). Use of platelet inhibitors was not associated with increased mortality. In conclusion, the use of warfarin before trauma was associated with increased 30-day mortality among a subset of patients with normal physiology on admission to hospital. Among patients with physiological derangement on admission, warfarin was not associated with increased mortality. Use of preinjury platelet inhibitors was not associated with increased mortality.

Paper IV

Thirty-five patients admitted to Oslo University Hospital Ullevål with ongoing GI-bleeding were consecutively recruited. Of these, 27 patients used drugs with potential antithrombotic effects. For comparison, treated controls without bleeding (13 on ASA and 11 on clopidogrel) and 27 healthy volunteers were recruited. Platelet function was measured by agonist-stimulated whole-blood aggregation and flow cytometry (P-selectin expression). Coagulation function was measured with calibrated automated thrombography. Platelet aggregation and P-selectin expression were significantly lower after arachidonic acid stimulation in GI-bleeding patients than in healthy subjects. Collagen-induced P-selectin expression was significantly reduced in patients using anti-platelet drugs and in many patients not using anti-platelet drugs. Thrombin generation, measured by calibrated automated thrombography, was only reduced in patients on warfarin treatment. In conclusion, platelet function was reduced
in acute GI-bleeding patients and a considerable proportion appeared to be related to drug use.

Paper V

The aim of the study was to assess whether corticosteroid use is associated with increased risk of GI bleeding or perforation. A systematic review and meta-analysis was performed to identify randomized, double-blind, controlled trials comparing corticosteroid treatment to placebo for any medical condition or in healthy participants. Studies with corticosteroids given locally, as single dose, or in studies with a crossover design were excluded. The databases MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews were searched between 1983 and 22nd May 2013. 159 studies, with 33 253 participants, were included. In total, 804 (2.4 %) patients had a gastrointestinal bleeding or perforation (2.9 % and 2.0 % for corticosteroids and placebo). Corticosteroids increased the number of GI bleeding or perforation by 40 % (odds ratio 1.43, 95% CI 1.22-1.66). The odds ratio was increased for hospitalised patients (odds ratio 1.42, 95% CI 1.22-1.66). For patients in ambulatory care, the increased odds ratio was not statistically significant (odds ratio 1.63, 95% CI 0.42-6.34). Only 11 GI bleeds or perforations occurred among 8651 patients in ambulatory care (0.13 %). Increased odds ratio was still present in subgroup analyses (studies with NSAIDs use excluded; odds ratio 1.44, 95% CI 1.20-1.71, peptic ulcer exclusion criterion excluded; odds ratio 1.47, 95% CI 1.21-1.78, and use of gastro-protective drugs excluded; odds ratio 1.42, 95% CI 1.21-1.67). In conclusion, corticosteroid use was associated with increased frequency of GI bleeding and perforation. This association was statistically significant for hospitalised patients only. For patients in ambulatory care, the total occurrence of bleeding or perforation was very low, and the increased occurrence of bleeding was not statistically significant.
5 DISCUSSION

5.1 Methodological considerations

In the following section the methodological aspects of the papers included in this thesis are discussed.

5.1.1 Study design

Randomized controlled trials are considered the gold standard in assessing drug effects. In contrast, the RCTs are usually underpowered and not designed to detect differences in (rare) adverse events between treatment groups [123]. Specific adverse effects such as intracranial bleedings or gastrointestinal bleedings occur quite infrequently, and may be defined as rare events. Therefore, for rare outcomes, for sub-populations, and in previously unrecognized adverse effects, studies with an epidemiological approach may be the only way to obtain reliable evidence [124]. Additionally, if there is a long latency between exposure and the adverse effect, RCTs may not be feasible and observational studies may be the only way to detect adverse effects. Examples of such studies are case-control studies, prospective or retrospective cohort studies, and meta-analyses. The spontaneous reporting system, the cornerstone in pharmacovigilance, may also be used to describe and follow trends in reporting patterns and to estimate deaths from adverse drug reactions [63, 87, 89, 90]. Since the epidemiological studies are of observational design, they are considered hypothesis generating. They may also be vulnerable to bias, since many of the epidemiological studies are designed to address a particular research question after the data are already collected (i.e., ad hoc hypothesis).

All the studies included in this thesis are retrospective and of observational design. The studies from the spontaneous reporting system (Paper I and II) and the trauma registry (Paper III) are retrospective cohort studies, while the study on ongoing GI bleeding (Paper IV) has a case-control design. Meta-analysis is considered of observational design, even when applied to RCT’s.

The spontaneous reporting systems (Paper I and II) are set up for signalling purposes. The very low reporting rate, even for adverse events which are compulsory to report, and poor-quality reports are the main limitation with the spontaneous reporting system [125-127]. Thus, the spontaneous reporting system cannot be used to estimate bleeding rates and frequencies of ADRs, due to both underreporting of adverse events and uncertainties around the number of patients exposed to the different drugs. Other limitations are reporting bias and incompleteness of follow up of patients.
Trauma registries (Paper III) are used to assess and improve patient care, for administrative purposes and research. Trauma data are prospectively collected by dedicated trauma registrars, however, errors at data collection and data entry can lead to incorrect information and skew study results [128]. The main limitation with the trauma registry at Oslo University Hospital Ullevål is the possibility of sampling bias as OUH-U is a Level 1 trauma centre for all the local hospitals in South-Eastern Norway. If patients on preinjury antithrombotic agents are less likely to be transferred from the local hospital to the trauma referral centre due to prospected poor outcome, this will introduce sampling bias in the trauma database, and our study may underestimate the true mortality risk of preinjury antithrombotic agent use.

In the study on ongoing GI-bleeding (paper IV) blood was drawn from study participants with the purpose of measuring platelet activity. Of the studies included in this thesis, this study was the only one in which a biological product was collected for the study purpose. During a period of two years 35 patients with ongoing gastrointestinal bleeding were included. This low number of patients was due to inclusion of patients during the weekdays between 09 a.m. and 02 p.m only, since platelet function tests have to be performed within three hours after blood has been drawn due to platelet reactivity. Platelet function tests, such as whole-blood aggregometry and flow cytometry are performed on specialised instruments not used in routine and operated by specially trained personnel. In addition, nurses on duty in the emergency department had to be informed of the study and remember the study to alert the study personnel to come and collect blood and informed consent from the patient. The study was performed at Oslo University Hospital Ullevål, one of the largest hospitals in Norway, and shows how long time it takes to collect patients for studies on adverse drug reactions.

Meta-analyses (Paper V) are performed to summarise results from several studies in order to aggregate information, achieve a higher statistical power for the measure of interest, and to resolve uncertainty when reports disagree. Both observational studies and RCT’s may be included in meta-analyses. To avoid the methodological problems with including observational studies, such as potential biases in the original studies, publication bias, and diversity of study designs and populations, only double-blind RCT’s was included as the data source in our systematic review and meta-analysis [129]. Double-blind RCTs are considered the “gold standard” of research on the basis of rigorous patient inclusion/exclusion criteria, a thorough monitoring and follow up of patients, restrictions of concurrent medications, and the heightened scrutiny of analysis and reporting. There are, however, several methodological pitfalls in using RCT’s on adverse events in meta-analyses [130, 131]. The main limitation is probably that adverse events are rarely the primary or secondary endpoint (or any endpoint at all) in a RCT, hence the quality and quantity of the reporting of adverse events in clinical trials is frequently inadequate [132]. Other limitations are selective publication and outcome(s)
reporting which can lead to distorted conclusions about adverse effects. Likewise, the study population included in a RCT may differ from real-life use of the particular drug, affecting the external validity of the meta-analysis.

5.1.2 Reporting bias

Underreporting, skewed reporting, and lack of data may be an issue in all types of studies. All papers included in this thesis were based on information that was already collected and meant for other purposes. The data sources were an external database (the Norwegian spontaneous reporting system database at The Norwegian Medicines Agency, Paper I and II), an internal database (the hospital based trauma registry at Oslo University Hospital Ullevål, Paper III), medical records (drug and laboratory data; Paper III and Paper IV), and RCT’s (Paper V).

In pharmacovigilance (Paper I and II), substantial underreporting and skewed reporting of adverse drug reactions is a well-known issue, and is probably the main limitation when data are collected from the spontaneous reporting system [126, 133, 134]. In addition, spontaneous reports often lack details, which makes analysis difficult. Since we cannot be sure if any of the patients had a bleeding ADR, whether the patients were exposed to the target drug(s) in question, or whether the drug(s) actually caused the bleeding ADR, the spontaneous reporting system cannot be used to estimate bleeding rates or frequencies of bleeding ADRs. Data from the spontaneous reporting system may, however, be used to provide insight into adverse drug effects in "real life" and the (inappropriate) co-prescribing of drugs [135].

In the head-trauma study (Paper III), there may be underreporting of antithrombotic drugs since this information is collected from patient records. Particularly drugs with pleiotropic effects on platelets and non-prescription drugs may be underreported in patient records. Since platelet effect was not measured, the magnitude of this bias is unknown. Therapeutic INR among five patients without documented warfarin use may also indicate underreporting of warfarin use, or trauma-induced coagulopathy. This counts to 1.8% of the patients without documented use of antithrombotic drugs (5/281), which can be considered as a relatively low underreporting rate.

In the study on ongoing GI bleeding (Paper IV) the main outcomes were platelet and anticoagulant activity with respect to use of antithrombotic drugs. Documentation of drug use was collected from the medical records. Thus, we cannot exclude underreporting of drugs, particularly over-the-counter drugs with effects on platelet function (e.g. NSAIDs).
In the meta-analysis (Paper V), lack of adverse events data in the primary publications (RCTs) was common since GI bleeding or perforation was not among the study outcomes. In our meta-analysis, trials were included if GI adverse events were documented or if assessment of adverse events monitoring were described in the methods section. This may, however, have introduced bias since the adverse event reporting was very variable between studies. Another main issue in the collection of GI adverse events data from the RCT’s, was the inconsistent definitions of GI adverse events between studies, i.e. from occult blood in stool to bleeding in need of blood transfusion. In addition, description of adverse events and presentation of data varied considerably. In some studies only adverse events with significant differences between the treatment groups were described. In other studies only adverse events occurring in a specific number of participants were described. Similar results, i.e. inadequate reporting of clinical adverse effects or laboratory toxicity, and lack of reasons for study discontinuations, has been documented in other medical areas, such as treatment of HIV, sinusitis, acute myocardial infarction, and hypertension [136, 137].

5.1.3 Measurement and instruments

Detection of adverse drug reactions requires patients’ and/or health care providers’ awareness of the event, and judgement about reporting the event. In addition, an assessment has to be performed or an instrument has to be used to detect or grade the adverse drug reactions.

All spontaneous reports reported to RELIS (Paper I and II) are coded according to drug and adverse event, in addition to a causality assessment. RELIS codes the adverse events into MedDRA terms. MedDRA is a coding system in which adverse events are coded according to a standardised medical terminology. The system is a hierarchical system that categorises medical terms into body-system organ classes. RELIS codes the adverse events according to the description in the case report. Bias may be introduced if the reports include only symptom terms or laboratory results. A causality assessment is used in order to standardise the classification of the likelihood the relationship between the suspected drug and the adverse drug reaction i.e. certain, probable, possible, unlikely, and unclassified [138]. As all cases and all drugs were included as recorded in the case reports in our analyses, some adverse drug reactions classified by RELIS as “unlikely” may have been included in the analyses. However, very few adverse reactions are classified as “unlikely” or “certain”; most are termed “probable” or “possible” according to a WHO document [138]. Thus, we suspect the misclassification rate to be low, but some bias may still have been introduced. In the published papers based on the data from the spontaneous reporting system (Paper I and II), we categorised drugs to have potential to contribute to bleeding and/or to interact with
warfarin. The primary source to identify drugs with potential to contribute to bleeding was Legemiddelhåndboken (The Norwegian National Formulary). Antithrombotic drugs, drugs with pleiotropic effects on platelets (i.e. NSAIDs and SSRIs), drugs with erosive effects on gastric mucosa, and cytotoxic drugs were included [139]. Other drugs with pleiotropic effects on platelets such as calcium antagonists, statins, angiotensin-converting-enzyme inhibitors (ACE inhibitors), and angiotensin II antagonists were not included since the association between these drugs and bleeding are less documented, and are not listed as interacting with warfarin. One or more of calcium antagonists, statins, ACE inhibitors, and angiotensin II antagonists are frequently used by patients on antithrombotic drugs due to concomitant cardiovascular /hypertensive /hypercholesteremic disease. Inclusion of those drugs would profoundly have inflated the numbers of drugs with potential to contribute to bleeding. The interaction chapters in the British National Formulary (BNF), Martindale and Legemiddelhåndboken were used to define drugs with potential to interact with warfarin. The three sources differed with respect to which drugs were listed as interacting. Particularly drugs acting pharmacodynamically with warfarin, such as antiplatelet drugs, were to a lesser extent listed in BNF and Martindale. Studies have shown an overall poor agreement between drug information sources in listings of drug-drug interactions. Olvey et al documented the agreement among three databases in assessment of critical drug-drug interactions to be as low as 13.7% [140]. Anthony et al have documented similar disagreement for warfarin interactions between FDA-approved label and three drug information compendia, as only 50 of 648 drug-interaction-pairs were listed in all four sources [69]. This low agreement may illustrate why different studies include different drugs as suspect and different drug interactions. We were interested in all warfarin-drug interactions and included both drugs acting pharmacodynamically and drugs acting pharmacokinetically with warfarin. Knowledge about interactions, however, is a dynamic field as new interacting drugs are steadily included and some are excluded as interacting with warfarin. This might make comparisons between studies over time difficult. As most drug interaction resources suggest that thyroid hormones, i.e. levothyroxine, can potentiate the effect of warfarin and predispose patients to bleeding [68], levothyroxine was included as potentially interacting with warfarin in our study on warfarin-associated bleeding events. In two recent studies this interaction has been disputed, as no occurrence of bleeding and no change in the warfarin/INR ratio were seen among patients on warfarin who were started on levothyroxine [141, 142].

Platelet function is not easily measured due to platelet reactivity. As a consequence, platelet function, except from platelet count, is only used for research purposes. In the study on ongoing GI bleeding (Paper IV), platelet function was measured. In contrast, no data on platelet function existed in the studies based on data from the spontaneous reporting system.
Use of platelet function tests could have indicated use of non-declared platelet inhibitors, particularly over-the-counter NSAIDs, and could have scrutinised platelet function effects when several drugs with inhibitory effects on platelets were used. In the study on ongoing GI bleeding, drugs with pleiotropic effects on platelets such as calcium antagonists, statins, ACE inhibitors, angiotensin II antagonists, beta blockers, and paracetamol were included as drugs with antithrombotic effects. The study was too small, however, to detect antithrombotic effects of single drugs.

Gastrointestinal bleeding is very concrete and is often easily detected and measured. All patients in the study on ongoing GI bleeding had clinical presentation of hematemesis and/or melena (Paper IV) and all case reports to the spontaneous reporting system (Paper I and II) included bleeding events, but of divergent severity and to different parts of the body. In the head-trauma study (Paper III), in contrast, we had no access to descriptions of intracranial pathology or cause of death. Such descriptions would have provided deeper insight to trauma severity and would have added further value to the results. The RCT’s included in our meta-analysis (Paper V) used several different definitions of bleeding, as hematemesis, melena, occult versus visible blood in stool, bleed in need of blood transfusion, or bleed in need of hospital stay. Such inconsistent definitions of adverse drug reactions may hinder data harmonisation, or, as we included all definitions, give diversity in the analysis [143].

Meta-analyses aim to provide an overall and objective measure of effect. There is, however, an amount of judgement in the acceptance or rejection of studies into the analysis and in the abstraction of data. This judgement may drastically change conclusions between meta-analyses. In our meta-analysis we aimed to have as few exclusion criteria as possible to enhance the generalisability of the results and since rejection of many studies could introduce further bias. Meta-analysis of all eligible studies may produce a precise estimate, but be seriously biased because of flaws in the conduct of some of the studies [144]. Thus, the Cochrane Collaboration recommends restricting meta-analyses to trials at low risk of bias or stratifying meta-analyses according to risk of bias. Other researchers have questioned this exclusion of trials with high-risk of bias, as treatment outcomes in meta-analyses has been show to differ depending on which studies are included-excluded, such as inclusion of all trials, inclusion of the single most precise trial, inclusion of the largest trials, adjusting for small-study effect, or inclusion of trials at low risk of bias only [145, 146]. Use of summary scores to assess trial quality is particularly problematic, as the type of scale used to assess trial quality could dramatically influence the interpretation of meta-analytic studies [147]. We included only randomized, double blind trials and assessed risk of bias by recording which methods were used for monitoring, definition and description of adverse effects,
randomization, and selection criteria. We did not perform sensitivity analysis according to risk
of bias, as recommended by Dechartres et al [145].

5.2 Discussion of main findings

5.2.1 Aim 1: Patterns of bleeding adverse drug reactions reported to the
Norwegian spontaneous reporting system

Paper I and Paper II document the patterns of all 616 bleeding adverse drug reactions
reported in Norway during a three year period from 2003 to 2005. Like previous years in
Norway, warfarin (289 reports) was the drug most often reported with fatal outcome. A study
from Sweden has shown similar results, with the vitamin K antagonists most often reported to
the spontaneous reporting system with fatal outcome [63]. In Sweden, during a ten year
period from 1995 to 2004, warfarin accounted for 387 of 990 (40%) of spontaneously
reported fatal ADRs [63]. Corresponding numbers from the United States (FDA Adverse
Event Reporting System) were 999 fatal bleedings (10 % of 9766 total bleeding cases) from
warfarin use during 1993 to 2006 [148]. Warfarin was also ranked among the top ten primary
suspect drugs having serious outcome. The number of inhabitants in the United States is
about 30 times higher than in Sweden. A 2.5 times higher number of reported warfarin
related deaths in the United States may indicate lower reporting rates, different compositions
of the population, lower incidence and prevalence of disease, different prescribing patterns,
or other factors. This illustrates why the spontaneous reporting system cannot be used to
estimate ADR rates and frequencies of ADRs. Warfarin has been among the drugs most
often reported with fatal outcome to the spontaneous reporting system in Italy and Canada
during the 1990s and 2000s, but 12 deaths in Italy and four deaths in Canada during five
years are very low numbers and do not allow for further analysis of the fatal reports or
analysis of drug interactions [87, 88]. In Germany, in contrast, heparin was the drug most
often reported as suspect of causing ADR during 1990-2002, and among the vitamin K
antagonists phenprocoumon was most often reported and was ranked as number 13 of
suspected drugs [90]. This indicates that phenprocoumon was preferred over warfarin in
Germany, and illustrates how different treatment traditions may affect ADR reporting and
make comparisons between countries difficult.

Breen et al published in 2003 a survey of all warfarin-associated adverse drug events
reported in Norway from 1990 – 2000 [62]. A main finding was the high occurrence of
bleeding during the first month of therapy (63% of the case reports), particularly during the
first 5 days of therapy (30% of the case reports). A Canadian cohort study from 2013 which
included 125 000 patients with atrial fibrillation who started warfarin therapy has confirmed
that the first 30 days of warfarin treatment conveys the highest risk of bleeding in clinical practice [149]. During the first 30 days, rates of haemorrhage were almost 12 % per person-year, which is considerably higher than the rates of 1 %-3 % reported in RCTs of warfarin therapy [46]. This illustrates the importance of performing studies in different patient populations to achieve knowledge of problems in real life use of drugs. In our study of warfarin-associated bleeding events (Paper I), although the first month of therapy conveyed the highest bleeding risk, the vast majority of bleeding events (74%) occurred after more than one year of warfarin use. Some of the bleeding events occurred after very long time (after more than 20 years on warfarin), indicating the need for close follow up of patients on warfarin even after start of therapy. We do not know the reason to the discrepancy between the two Norwegian studies on warfarin-associated bleeding events, but doctors might have been more cautious in the initiation phase of warfarin therapy due to the paper by Breen et al published in Tidsskriftet, or, knowing that the start of therapy as a high risk period, report the late bleeding events as unsuspected.

However, the majority of the bleeding events reported to the Norwegian spontaneous reporting system during 2003-05 were not associated with warfarin use. The 327 non-warfarin-associated bleeding events (Paper II) comprised 53% of all case reports on bleeding ADRs, which included use of fibrinolytics, heparins, platelet inhibitors (acetylsalicylic acid, NSAIDs/Cox-2 inhibitors, clopidogrel, dipyridamole, and antidepressants), and immunosuppressants alone or in combinations before bleeding. Duration of treatment, indication for drug use, and drug doses were rarely reported in the non-warfarin-associated bleeding events and could not be used for analysis.

The majority of all bleeding events reported in Norway were characterised by the reporter as serious (90%), but the warfarin-associated and the non-warfarin-associated bleeding events varied with respect to bleeding locations and mortality. Most warfarin-associated bleeding events were cerebral (60%), which was reflected in a high mortality (48%). The non-warfarin-associated bleeding events, in contrast, had a much lower mortality rate (21%), mostly due to fewer cerebral bleeds (17%) and more gastrointestinal (58%) and other bleeds (25%). This is consistent with the mortality rates seen in observational studies [42-44]. Surveys of spontaneous reporting of adverse drug reactions have also been performed in France and Portugal, but both drugs and ADRs were described according to the organ or system on which they act, making comparisons with the Norwegian reports impossible [89, 150].

The majority of the bleeding events occurred among elderly people, with a mean age of 76 years in Paper I and a median age of 74 years in Paper II. The results are consistent with the study by Breen et al (median age 75 years) and Wester et al (median age 76 years) [62, 63].
Paper I and Paper II further document the extent of polypharmacy at the time of bleeding. Among the warfarin-associated bleeding events, the median number of drugs was 4.0 per patient (range 1-17) and among the non-warfarin-associated bleeding events it was 3.0 per patient (range 1-18). Polypharmacy has been shown to be a risk factor of bleeding among warfarin users [72]. In a recent Swiss study 50% of the elderly patients with venous thromboembolism used more than four different drugs, and this polypharmacy was associated with increased risk of bleeding [151]. There is limited documentation of polypharmacy among bleeding events other than warfarin.

In Norway, from 2007 onwards the number of reported warfarin-associated adverse events has decreased (see Figure II), and from 2013 as the novel oral anticoagulants (NOACs) have come to the market, NOACs are most often reported with fatal outcome [7]. This shows how treatment changes now are reflected in the reported adverse events.

![Figure II: From Årsrapport bivirkninger 2011, Seksjon for legemiddelovervåkning, Statens legemiddelverk. Figure II shows number of reported adverse events and the number of deaths reported to the Norwegian Medicines Agency.](image-url)
5.2.2  **Aim 2: To examine the extent of potential drug interactions in the spontaneous reports and concordance in assessments between reporters and evaluators**

In Paper I and Paper II the extent of potential drug interactions were assessed. Documentation of potential warfarin-drug interactions in 51% of the warfarin-associated bleeding events is consistent with other studies, showing that potentially interacting drugs were prescribed for 40-80 % of the anticoagulant users [70-74, 80]. Warfarin use is hampered with the potential of drug interactions. Together with a narrow therapeutic range, difficulty of dosage adjustments, a substantial bleeding risk, and the fact that drug interactions are often preventable, studies have been performed to scrutinise the occurrence of warfarin-drug interactions. As studies from the spontaneous reporting system on warfarin-drug adverse events are very limited, existing studies mostly come from other registries. Many studies from pharmacy or prescription databases use number of patients who are prescribed potentially interacting drugs as the main outcome. Most of such potential drug-drug interactions, however, have been described as clinically insignificant, as interactions with severe outcome are rare [80]. In studies based on data from the General Practice Database in the United Kingdom and a Swedish hospital registry, in contrast, the extent of drugs interacting with warfarin was described for patients suffering a bleeding event. In the study by Gasse et al, 62 % of cases (intracranial, GI, epistaxis, other bleeding sites) were exposed to potentially interacting drugs compared to only 36 % of the controls without bleed [72]. In the study by Jonsson et al, which included patients with cerebral haemorrhage only, 41 % of patients on warfarin used potentially interacting drugs [74].

In Paper II, two or more drugs with potential to cause bleeding were documented in 45% of the included ADR reports. How this compares to other populations is not known, as less focus has been on antithrombotic drug interactions, except from vitamin K antagonists, due to fewer problems with (pharmacokinetic) drug interactions. In a review article, Magro et al summarise the current knowledge of ADRs caused by drug-drug interactions [152]. The occurrence of drug-drug interactions associated with ADRs reported to the spontaneous reporting system varied from 1% to 35%. The included studies, however, focused on specific drug-drug interaction pairs and did not focus on a particular outcome (bleeding) as we did. In paper II (non-warfarin-associated bleeding events) we used a logistic regression model to detect factors associated with mortality, as we suspected that use of more drugs with effect
on haemostasis could have additive or synergistic effects. Surprisingly, number of drugs with effect on haemostasis was not associated with mortality, probably reflecting that these drugs have highly variable bleeding risk. Weighted effects of each drug might have been a better predictor of mortality than number of drugs affecting haemostasis. Number of drugs used, in contrast, was associated with mortality in the regression analysis. As number of drugs (polypharmacy) may reflect co-occurrence of two or more chronic medical conditions (multimorbidity) more than the number of drugs per se, the association may not be causal as number of chronic medical conditions is associated with increased mortality.

In Paper I we documented differences between short term (< 3 months) and long term (> 3 months) warfarin users with respect to interacting drugs and INR. As suspected, patients using drugs interacting pharmacokinetically with warfarin had higher INR than patients using warfarin alone or together with pharmacodynamically interacting drugs, but this was valid only for late bleeding events. In the early bleeding events, in contrast, patients using warfarin alone had a very high INR, suggesting that pharmacogenetic polymorphisms might have contributed to over-dosing and bleeding. Pharmacogenetic polymorphisms have been associated with an increased risk of over-anticoagulation and of bleeding events among patients in a clinic setting [153, 154].

In Paper I and Paper II we documented a substantial discrepancy between reporters and evaluators in assessments of drug interactions. Most reporters assessed one drug as suspect of bleeding only. In contrast, two or more drugs with potential to cause bleeding were documented in about half of the included ADR reports and were assessed as interacting by the evaluators. As suspected, there was a higher degree of under-reporting of pharmacokinetically than of pharmacodynamically interacting drugs in the warfarin-associated bleeding reports. Drugs interacting pharmacokinetically with warfarin are not intuitive, and must often be looked up in drug interaction compendia. Pharmacodynamic interactions, in contrast, are more intuitive due to the antithrombotic effects of the drugs. In Paper II drugs with effect on haemostasis, or with antiplatelet effect, erosive effect, or cytotoxic effect were considered as potentially interacting as all are associated with increased bleeding risk. The results showed a significant difference between reporters and evaluators in assessment of drug-drug interactions, as reporters to a lesser extent suspected heparin and platelet inhibitors other than NSAIDs/COX-2 inhibitors. This difference is important to recognise if studies using data from the spontaneous reporting system draw conclusions based on the reporters’ evaluation only. The ability of prescribers to recognise potential drug-drug interactions is low [155, 156]. Recently, computerised drug alert systems have become available to Norwegian primary care physicians to help prevent drug-drug interactions, and internet based drug-interaction checkers are easily available. In future
spontaneous reports, one might expect fewer bleeding events due to unintended drug interactions. The spontaneous reporting database could be a valuable resource for detection of reporters’ knowledge of drug-drug interactions, however, to our knowledge only one study has previously considered this issue [85]. In a study based on case reports from the Italian spontaneous reporting system, all reports containing at least two drugs reported as being suspect of causing the ADR or as concomitant medication were included. For each report containing a potential drug-drug interaction, it was verified whether the description of the ADR corresponded to the drug-drug interaction effect. In 20% of the reports, both interacting drugs were recognised as suspect by the reporter, which corresponds to our finding of 17% in our study of warfarin-associated bleeding events.

In Paper II, we searched for potential new ADR signals using VigiMine, a programme providing statistical data whether a particular drug-ADR combination occurs more often than expected when considering all the reports in the WHO database. Of the 33 drugs/reports suspected of being associated with bleeding by reporters only and without any explanation for the bleeding event, three drug/ADR combinations came out with positive signal. The drug/ADR combinations however, have previously been described and none represents new signals.

5.2.3 Aim 3: Preinjury antithrombotic drugs and mortality after head trauma

In paper III, use of warfarin before trauma was associated with increased mortality among a subset of patients with normal physiology on admission. Among patients with physiological derangement on admission, both warfarin users and non-users had a high mortality. Platelet inhibitor users, in contrast, had a low mortality, not significantly different from non-users.

Our findings of different mortality between different subsets of patients on warfarin, is in accordance with some previous studies [96, 102, 157]. Two of these studies are very large trauma database studies, with impressing 130 000 patients and 1 200 000 patients with trauma, respectively, included [96, 102]. Both studies included patients of younger ages and patients sustaining non-head traumas, i.e. patients with lower expected mortality. The study by Lecky et al included 499 patients on warfarin of whom 212 sustained head trauma. The study by Dossett et al included 36 000 patients on warfarin of whom 1400 were both older than 65 years and sustained head trauma, compared to 57 patients 55 years and older on warfarin in our study. When subgroup analyses were performed in the large database studies, however, warfarin was not associated with increased mortality among patients older than 65 years (24 % mortality among warfarin users compared to 23 % among nonusers) [96], and the difference in mortality between warfarin users and nonusers with head injury was not significant (38 % and 35 %, respectively, odds ratio 1.41, 95% CI 0.90-2.21) [102]. These
numbers are comparable to the mortality documented in our study among patients with physiological derangement (35% mortality among warfarin users and 32% among nonusers). It seems that the negative impact of preinjury warfarin use on mortality is most pronounced in patients with normal physiology, defined as T-RTS = 12, on admission. Howard et al has previously described similar results [157]. They did not use T-RTS, however, but a combination of an anatomical trauma score (AIS head) and a physiological trauma score (GCS). In addition they grouped GCS 15 with GCS 14. GCS 14 was considered as physiological derangement (T-RTS≤11) in our study.

Such large databases studies as by performed by Dossett et al and Lecky et al, enable the researchers to include most of the cases in a nested case-control analysis, which is considered a better study design than a retrospective cohort analysis. It may be considered more efficient to control for the effects of age, gender, anatomical and physiological severity, and comorbidity at the design stage, rather than controlling for their potential confounding effects when the data are analysed. In our study, however, we would not have detected a difference in mortality between patients with normal physiology and physiological derangement on admission if we had matched cases with controls on trauma severity.

The documentation whether preinjury use of antiplatelet agents affects mortality after head trauma is even less clear than for warfarin. Our study is consistent with most of the few previously published studies in that no effect on mortality was documented [100, 108, 109]. Most studies on antithrombotic agents do not distinguish between different antiplatelet agents such as acetylsalicylic acid and clopidogrel. If clopidogrel is conveyed with higher risk of bleeding, pooling of antithrombotic agents might conceal its true bleeding risk. A recent meta-analysis on the effect of preinjury antiplatelet agents on mortality in patients with blunt head trauma concluded with insignificant increased risk for both acetylsalicylic acid (odds ratio 2.4, 95% CI 0.6-9.3) and clopidogrel (odds ratio 1.5, 95% CI 0.3-7.5) [111]. The meta-analysis was, however, performed on observational studies and differences in age and trauma severity were not adjusted for in the meta-analysis. Additionally, the meta-analysis included only four studies in each group, the included studies had few participants each and they had very divergent odds ratios of mortality, indicating that these results are not robust.

5.2.4 Aim 4: Platelet activity in patients admitted to hospital with acute GI bleeding

Reduced platelet function in patients with ongoing GI bleeding was documented in Paper IV and a considerable portion appeared to be related to drug use. Many studies have focused on platelet function at start of therapy or platelet function in patients with thrombotic events or
undergoing coronary intervention, i.e. treatment resistance [158]. Our study, in contrast, was focused on reduced platelet function as an adverse effect. The patients in our study used several drugs with antithrombotic effects, but due to a small sample size and several drug combinations subgroup analyses were not possible to perform. There are limited data regarding concomitant use of several antithrombotic drugs as most studies focus on single drugs and ignore use of drugs with pleiotropic effects on platelets. Pasa et al measured platelet activity in patients with upper GI bleeding [159]. Of 49 patients on ASA or a NSAID, 73.5 % showed reduced platelet function measured as arachidonic acid- or ADP-induced aggregation. The included patients used ASA or NSAIDs for pain or rheumatic diseases. Patients with cardiovascular diseases were excluded. Use of concomitant medication was not accounted for. More recently, Barinov et al analysed platelet aggregation in patients with GI ulcer bleeding [160]. Blood was sampled at the time of hospital admission. Drug use was recorded. Of 247 included patients 71 % had a previous history of peptic ulcer disease and 30 % received NSAIDs or ASA. Use of other drugs was, in contrast, not described. Main outcome was platelet aggregation according to sustainable or unsustainable haemostasis, not according to use of platelet inhibitors. The study is therefore not comparable to our study. In addition, the population are probably different than ours as fewer patients included in Barinovs study used antiplatelet drugs compared to our study (30 % vs 50 %, respectively). Van der Mejden et al has assessed platelet function in a case-control study of patients treated with warfarin and recurrent bleeding [161]. There was no difference between patients and controls in arachidonic acid- or ADP-stimulated aggregometry, however, platelets from two controls and one patient did not respond to arachidonic acid, which could later be attributed to intake of ASA. No difference was measured between patients and controls in flow cytometry analyses. According to the published paper, patients and controls were retrospectively identified and invited to participate in the study. The paper did not state whether blood was collected at the time of bleeding or later on, when the patients did not bleed.

5.2.5 Aim 5: Corticosteroids and GI bleeding or perforation

Paper V documents an association between corticosteroid use and increased occurrence of GI bleeding compared to placebo. A similar increased occurrence of gastric / peptic ulcer, GI bleeding or perforation has been shown in previous studies, but since these studies are smaller, the results have not always reached statistical significance [27, 119, 121, 162]. Therefore, some authors have concluded with a non-association between use of corticosteroids and GI bleeding [26, 30, 163]. The estimated relative risks across studies, however, vary from about 1.1 (non-significant) to 1.5 (marginally significant) [164].
Conn and co-workers performed the first meta-analysis in 1976 [30]. They found that in double blind studies ulcers occurred slightly more frequently among patients treated with corticosteroids compared to placebo, but as the differences were non-significant, they concluded with a non-association between corticosteroid use and GI adverse events. Messer et al, in contrast, reached an opposite conclusion in 1983 as they found a relative risk of 2.3 and 1.5 for peptic ulcer and GI bleeding, respectively [27]. Later, Conn and Poynard did a critical reanalysis of the data by Messer et al after supplementary information had been collected from the authors of the included papers [163]. An insignificant relative risk of 1.3 was documented. Piper et al found an estimated risk of only 1.1 with corticosteroid use alone in a nested case-control study in 1991 [115]. In the latest study published in 1994 by Conn and Poynard, analyses indicated odds ratios from 1.1 (gastric or duodenal erosion) to 4.8 (perforation of lower intestinal viscera) [26]. More recent updates have only been performed for single diagnoses as part of Cochrane reviews to determine relative benefits of corticosteroids. What all previous studies have in common, is too few study participants and too few adverse events to get statistically significant results, given an estimated 30-40% increased risk of GI adverse events with corticosteroid use.

The longstanding dispute between Conn and Messer highlights several important dilemmas and pitfalls in performing systematic reviews and meta-analyses on adverse events. Firstly, there was a tendency of higher bleeding risks in single-blind studies compared to double-blind studies as well as alterations of results according to inclusion or exclusion of studies to the meta-analysis. This emphasizes how study design and assessment of studies may profoundly change the result. We included double-blind randomized controlled trials only, and had few other exclusion criteria with the aim to increase the generalisability. Our meta-analysis thus included 159 studies comprising 33,000 participants and got statistical significant results for the main outcome. We lost statistical significance in several subgroup analyses; however, there were consistently more GI adverse events in the corticosteroid groups than the placebo groups, indicating robustness of the results. Secondly, as most RCTs are not designed or powered to detect adverse events, data for meta-analyses often have to be collected across diverse patient groups. In a meta-analysis of adverse events, inclusion of diverse patient groups is often done, assuming that different diseases do not affect the risk of the adverse event. This assumption may not be correct. In our analysis, there was a substantial difference in events per 1000 patients between hospitalised patients and patients treated ambulant, indicating there may be a drug-disease interaction. In addition, different and inconsistent definitions of adverse events between studies may make comparisons difficult. Conn and Poynard reported the GI adverse events as exacerbated/new gastric/duodenal/unknown ulcers, haemorrhage/perforation/death from ulcer, totally 9
analyses. They did not do a pooling of a composite endpoint i.e. any GI adverse event. This highlights how unwanted (adverse) effects might “vanish” by performing subgroup analyses only. We pooled our endpoints as we assumed a perforation might have been an unrecognised GI bleeding.

We did not collect data on corticosteroid doses, cumulative drug doses, or duration of therapy. If severely ill patients were given higher doses and less ill patients were given lower doses of steroids, our results could be a result of higher doses instead of disease severity. We neither did subgroup analysis according to treatment year. Since eradication of the bacterium Helicobacter Pylori by triple therapy became standard treatment of gastritis or gastric ulcer from 1994, this may have affected GI adverse effects by corticosteroid use.

Our results contribute to the knowledge of GI adverse events associated with the use of corticosteroids. Although such post-hoc analyses cannot ascertain causality and are only hypothesis generation, no RCT large enough to establish causality regarding steroids and GI bleeding will probably ever be performed. The knowledge has to rely on RCTs performed for other purposes than adverse event analysis and observational studies. A significant risk of GI bleeding or perforation by corticosteroid use may, however, not be taken as a significant clinical risk. Compared to the increased bleeding risk seen with NSAIDs and ASA, the increased risk documented in our study may be considered small. It must be up to the clinician to decide, guided by our results and subgroup analyses, how the knowledge should be used [165, 166].
6 CONCLUSIONS

- Warfarin-associated bleeding events reported to the Norwegian spontaneous reporting database were associated with a high occurrence of cerebral bleeds and a high mortality. Non-warfarin-associated bleeding events were associated with a high occurrence of gastrointestinal bleeds and had a lower mortality (Paper I and II).

- Two or more drugs with potential to cause bleeding were documented in about 50% of the bleeding events reported to the Norwegian spontaneous reporting database. There was a substantial under-reporting of suspected drugs by the reporters (Paper I and II).

- Use of warfarin before head trauma was associated with increased 30-day mortality among patients with normal physiology on admission to hospital, but not among patients with physiological derangement on admission. Preinjury use of antithrombotic agents was not associated with increased mortality (Paper III).

- Platelet function was reduced in patients with ongoing GI bleeding and a considerable proportion appeared to be related to drug use (Paper IV).

- Corticosteroid use was associated with a 40% increased risk of gastrointestinal bleeding or perforation. The increased risk was statistically significant for hospitalised patients only. For patients in ambulatory care, the total number of bleeding events was very low, and the increased risk was not statistically significant (Paper V).
7 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

Drug-induced bleeding is a considerable health problem, hence much effort should be done to detect, describe, categorise and assess different aspects of the bleeding events to prevent morbidity and mortality. We have used different study designs and different patient populations to describe and assess different aspects of drug-induced bleeding events. As all our included studies have data collected for other purposes, the results may be vulnerable to risk of bias. And, as all the included studies are considered observational, no causality can be established. Our studies, however, have showed that the ultimate study to detect or assess drug-induced bleeding does not exist but will depend on the research question, the occurrence of the adverse event and the available resources.

How should adverse event data be collected in the future?
Prospectively collected adverse event data could potentially give further insight into adverse drug effects than the retrospective studies included in this thesis. Given a well-defined patient population with regular health care contact and health care professionals with knowledge of and interests in adverse drug reaction monitoring, adverse effect data could be collected more systematically than in any of the studies included in this thesis. Such monitoring programmes or active surveillance programs are gaining ground, as the data on adverse effects could be collected faster and more systematically. For rare events and in RCTs to treat adverse events, however, prospective collection of adverse events may be very time consuming and may not be feasible, as has been shown in a hypothetical RCT to treat warfarin-associated intracerebral haemorrhage [167].

The bleeding events from the Norwegian spontaneous reporting database were reported between 2003 and 2006. More lately, drug interaction checkers and automated drug alerts have been implemented in the computer software programs at the pharmacies and in the electronic prescription systems. Computer software programs have been suggested as the solution to drug-drug-interaction problems. Although the programs have unquestionable advantages, they also have important limitations, with variable accuracy, and inconsistently severity ranking. With the implementation of such software programs, however, we would expect the occurrence of i.e. warfarin-drug interactions to subside. Additionally, as use of warfarin is decreasing, we would expect a lower incidence of cerebral bleeds associated with warfarin use or with warfarin-drug interactions. However, as warfarin is replaced with novel oral anticoagulants (NOAC), new and unforeseen adverse effects will emerge. The future will need the pharmacovigilance system, in addition to more systematic reporting, use of patient administrative and medical records databases, use of systematic reviews and meta-analysis of adverse events, and even patient reporting.
8 REFERENCES


47. Rothwell, P.M., External validity of randomised controlled trials: "to whom do the results of this trial apply?". Lancet, 2005. 365(9453): p. 82-93.


