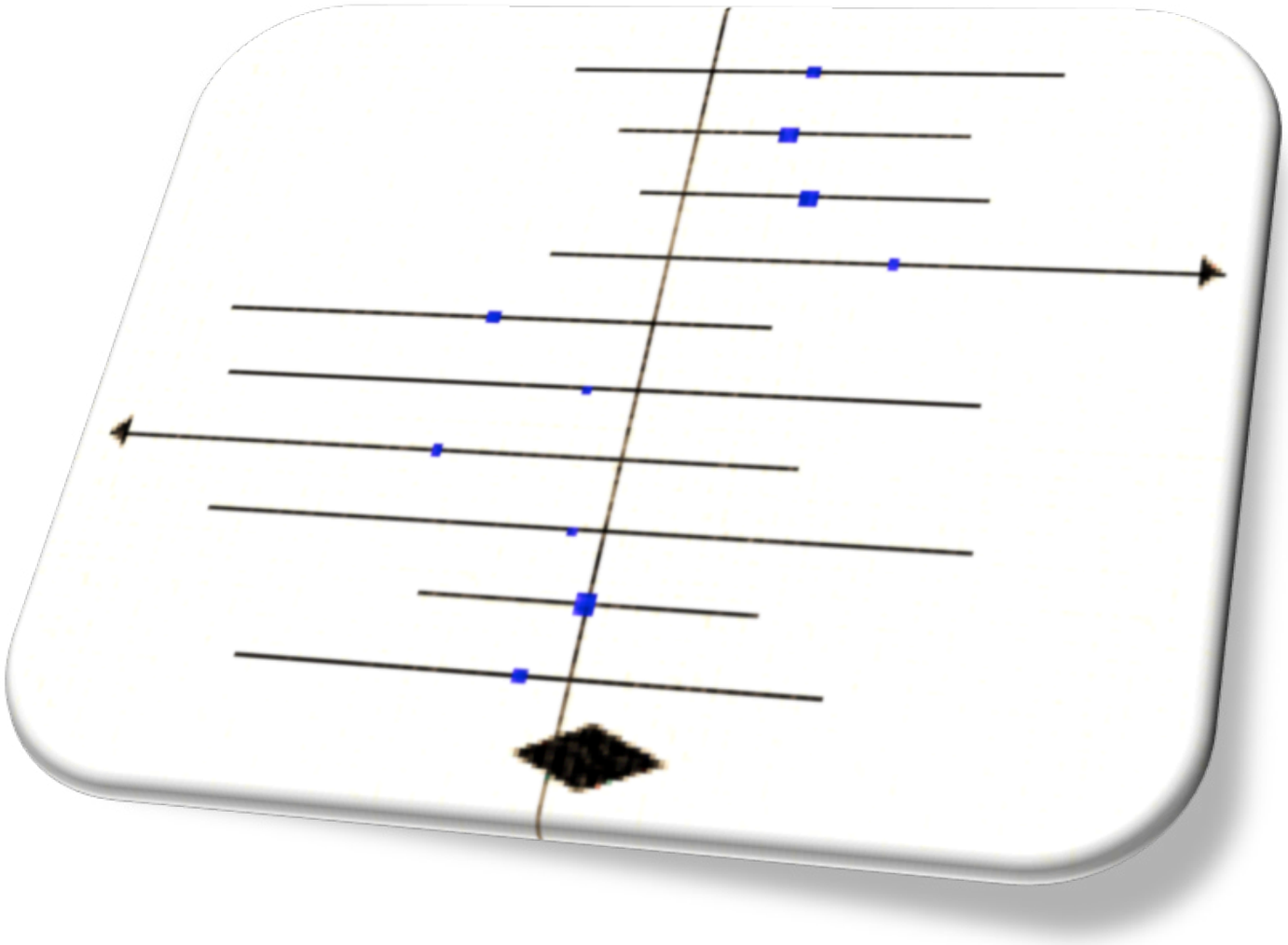

Carlos Miguel Costa Alves

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Tese de doutoramento em Ciências Farmacêuticas, especialidade de Farmácia Clínica, orientada pela Professora Doutora Ana Filipa Pereira Amaral de Macedo e pelo Professor Doutor Francisco Jorge Batel Marques e apresentada à Faculdade de Farmácia da Universidade de Coimbra

Maio 2014

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All the research work presented in this thesis was performed in strict collaboration of the Health Sciences Research Centre, Faculty of Health Sciences, University of Beira Interior, the Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra and the Centre for Health Technology Assessment and Drug Research, Association for Innovation and Biomedical Research on Light and Image, under the supervision of Professor Francisco Jorge Batel Marques and Professor Ana Filipa Pereira Amaral de Macedo and funded by Portuguese Foundation for Science and Technology (SFRH/BD/64957/2009).

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LIST OF ABREVIATIONS

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ADA	American Diabetes Association
ADR	Adverse drug reaction
ADVANCE	Apixaban Dose Orally vs. Anticoagulant with Enoxaparin
AE	Adverse event
AHRQ	Agency for Healthcare Research and Quality
APROPOS	Apixaban PROphylaxis in Patients undergoing tOtal knee replacement Surgery
ATC	Anatomical Therapeutic Chemical
BCPNN	Bayesian Confidence Propagation Neural Network
BID	Twice-daily
BMJ	British Medical Journal
CDER	Centre for Drug Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CIOMS	Council for International Organizations of Medical Sciences
CMDh	Coordination Group for Mutual Recognition and Decentralised Procedures
CONSORT	Consolidated Standards of Reporting Trials
CPRD	Clinical Practice Research Datalink
CRISP	Computer Retrieval of Information on Scientific Projects
DARE	Database of Abstracts of Reviews of Effects
DSRU	Drug Research Unit
DURATION	Diabetes Therapy Utilization: Researching Changes in A1C, Weight and Other Factors Through Intervention with Exenatide Once Weekly
DVT	Deep venous thrombosis
EADS	European Association for the Study of Diabetes
EBM	Evidence-Based Medicine
ECs	Ensaios clínicos
EMA	European Medicines Agency
EMEA	European Agency for the Evaluation of Medicinal Products
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
EPAR	European Public Assessment Report

ERMS	European Risk Management Strategy
EU	European Union
FDA	Food and Drug Administration
FDA-AERS	FDA's Adverse Event Reporting System
GLP-1	Glucagon-like peptide-1
GP	General practitioner
HLGT	High-Level Group Term
HR	Hazard ratio
ISOP	International Society of Pharmacovigilance
ISPE	International Society on Pharmacoepidemiology
JAMA	Journal of American Medical Association
LEAD	Liraglutide Effect and Action in Diabetes
LMWHs	Low molecular weight heparins
MAHs	Market Authorisation Holders
MedDRA	Medical Dictionary for Regulatory Activities
MEDLINE	Medical Literature Analysis and Retrieval System Online
MOOSE	Meta-analysis Of Observational Studies in Epidemiology
OR	Odds ratio
PE	Pulmonary embolism
PEM	Prescription-Event Monitoring
PhVWP	Pharmacovigilance Working Party
PPA	Prescription Pricing Authority
PPD	Prescription Pricing Division
PRAC	Pharmacovigilance Risk Assessment Committee
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
PRR	Proportional Reporting Ratio
QUOROM	Quality of Reporting of Meta-analyses
QW	Once-weekly
RCT	Randomized clinical trial
RD	Risk difference
RECORD	Regulation of Coagulation in Orthopedic Surgery to Prevent Deep Venous Thrombosis and Pulmonary Embolism
RevMan	Review Manager
RR	Relative risk
SE	Standard error
SIGLE	System for Information on Grey Literature in Europe

TGA	Therapeutics Goods Administration
TSR	Targeted Spontaneous Reporting
US	United States
US Congress	United States of America Congress
USA	United States of America
VTE	Venous thromboembolism
WHA	World Health Assembly
WHO	World Health Organization

PUBLICATIONS

PUBLICATIONS

Full text publications

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Data sources on drug safety evaluation: a review of recent published meta-analyses. **Pharmacoepidemiol Drug Saf.** 21:1 (2012) 21-33.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Apixaban and Rivaroxaban Safety After Hip and Knee Arthroplasty: A Meta-Analysis. **J Cardiovasc Pharmacol Ther.** 17:3 (2012) 266-276.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. **Diabetes Res Clin Pract.** 98:2 (2012) 271-284.

ALVES, C.; MACEDO, A.F.; MARQUES, F.B. – Sources of information used by regulatory agencies on the generation of drug safety alerts. **Eur J Clin Pharmacol.** 69:12 (2013) 2083-2094.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Drug-safety alerts issued by regulatory authorities: usefulness of meta-analysis in predicting earlier risks. **Eur J Clin Pharmacol.** 2014 DOI:10.1007/s00228-014-1670-5.

ABSTRACT/RESUMO

ABSTRACT

The assessment of the benefit/risk relation is conducted throughout the entire drug life cycle. Before a market authorization is granted, randomized clinical trials are designed to evaluate the efficacy and safety of a drug in a specific therapeutic indication. These studies are able to detect the most frequent adverse events. However, rare and/or long-latency harmful events are usually detected after a drug becomes available in the market. The increased seriousness of some adverse events may lead to label update with warnings or even to a drug withdrawal after being marketed for some years. Post-marketing observational studies may better reflect the nature of adverse events occurring in clinical practice since they include populations usually underrepresented in clinical trials, such as the elderly, pregnant women or patients with comorbidities.

The investigation of uncommon or long-term adverse events associated with pharmacological interventions has been discussed as a potential important application of meta-analysis. Meta-analysis is a systematic approach to synthesize and combine the results of selected studies. It is used to identify sources of variation among study findings and to provide an overall measure of effect to reach conclusions about a body of research. The meta-analytic technique has been applied with increasing frequency to clinical trials when efficacy assessments are needed. Although not frequently, meta-analysis conducted for safety purposes have also found increased risk estimates associated with some drugs, such cardiovascular adverse events due to rosiglitazone.

This project carried out in order to identify the role of meta-analysis as a Pharmacovigilance approach and to evaluate how best to combine safety information from both experimental and observational studies through this statistical technique. Only a limited number of meta-analyses are currently devoted to evaluate drug safety as a primary outcome. Of these, very few combine data from both observational and experimental studies. Although statistical significant risk estimates could be reached with the inclusion of observational studies in meta-analysis, isolated or in combination with clinical trials, the increased between-studies heterogeneity usually associated may preclude any definitive conclusions.

Authorities do not rely solely on risk estimates produced by meta-analysis and usually review additional sources of information to support benefit/risk ratio reevaluations due to safety issues. It was also demonstrated that cumulative meta-analysis was able to estimates increased iatrogenic risks years earlier than regulatory decisions have been taken by

authorities for the corresponding safety issues. However, excessive heterogeneity resulting from different study designs included in these set of meta-analyses may be one of the reasons delaying the acceptance of this technique by regulatory authorities when evaluating medicines safety profile.

Although reliable risk estimates have shown to be produced from meta-analyses conducted to evaluate drug safety issues, between-studies heterogeneity may not let drawing robust conclusions from those results, in particular when observational studies are included. The results of this work do not let recommend that a meta-analysis of the existing evidence should be conducted whenever a safety alert is issued. Moreover, this technique does not replace further assessments when the benefit/risk ratio profile of a medicine needs to be revised due to safety issues.

RESUMO

A relação benefício/risco de um medicamento é avaliada durante todo o seu ciclo de vida. Antes de ser concedida a autorização de introdução no mercado, a eficácia e a segurança de um medicamento numa determinada indicação terapêutica são avaliadas através da condução de ensaios clínicos aleatorizados e controlados. Estes estudos são capazes de identificar a maioria dos eventos adversos associados ao tratamento com um medicamento. No entanto, após a introdução no mercado, eventos adversos raros e/ou de longo tempo de latência podem ocorrer durante o tratamento com um determinado medicamento, sem que antes tenham sido identificados durante o desenvolvimento clínico. Os estudos observacionais pós-comercialização permitem identificar eventos adversos raros e/ou de longo tempo de latência. A gravidade acrescida de alguns eventos adversos pode levar à inclusão de uma advertência no resumo das características do medicamento ou até mesmo à sua retirada do mercado. Os estudos observacionais pós-comercialização podem refletir melhor a natureza dos eventos adversos que ocorrem durante a prática clínica, uma vez que estes estudos permitem avaliar subpopulações de doentes que não são frequentemente incluídas nos ensaios clínicos, como os idosos, as grávidas ou as crianças.

A investigação do risco de ocorrência de eventos adversos raros e/ou de longo tempo de latência associados ao tratamento com intervenções farmacológicas tem vindo a ser discutida como uma potencial aplicação da meta-análise. A meta-análise é uma ferramenta estatística que permite sintetizar e combinar resultados de vários estudos. É utilizada para identificar causas para a variação dos resultados entre os estudos e permite obter uma medida de efeito. A meta-análise tem sido aplicada frequentemente a ensaios clínicos com o objetivo de conduzir avaliações da eficácia das intervenções. Embora menos frequentemente, também têm sido conduzidas meta-análises de segurança que em alguns casos identificaram riscos acrescidos para eventos adversos, como o risco acrescido de eventos cardiovasculares associado à rosiglitazona.

Este projeto foi conduzido com o objetivo de identificar o papel da meta-análise na Farmacovigilância e para avaliar como combinar diferentes fontes de informação sobre segurança, nomeadamente estudos experimentais e observacionais, através da técnica meta-analítica. Apenas uma pequena proporção das meta-análises conduzidas atualmente considera a segurança como marcador primário. Destas, muito poucas combinam informação de estudos experimentais e estudos observacionais. Apesar das meta-análises que integram informação de estudos observacionais, de forma exclusiva ou em combinação

com estudos experimentais, poderem produzir estimativas de risco estatisticamente significativas, a elevada heterogeneidade que normalmente lhes está associada não permite que se tirem conclusões definitivas com base nesses resultados.

As autoridades reguladoras não baseiam as suas decisões apenas nos resultados produzidos pelas meta-análises quando pretendem conduzir reavaliações da relação benefício/risco dos medicamentos devido a questões de segurança, e têm em conta a informação gerada por outros estudos. Os resultados obtidos durante este trabalho demonstraram também que a integração cumulativa dos resultados de vários estudos através da meta-análise permitiu estimar riscos acrescidos para o desenvolvimento de eventos adversos associados a medicamentos para os quais as autoridades tomaram decisões regulamentares posteriormente à data em que se alcançou essa estimativa. No entanto, a excessiva heterogeneidade que resultou da inclusão de estudos com diferentes delineamentos nestas meta-análises pode ter sido uma das razões que tem impedido uma melhor aceitação desta técnica pelas autoridades reguladoras.

Apesar de a meta-análise poder produzir estimativas de risco fiáveis quando se avaliar a segurança de medicamentos, a heterogeneidade excessiva que se verifica em alguns casos pode impedir os investigadores e as autoridades reguladoras de avaliar corretamente a relação causa-efeito entre a exposição ao medicamento e o evento adverso, particularmente quando se incluiem estudos observacionais. Os resultados deste trabalho não permitem recomendar que se conduza uma meta-análise sempre que ocorra um alerta de segurança. Desta forma, a meta-análise não substitui outras fontes de informação quando a relação benefício/risco dos medicamentos necessita de ser reavaliada devido a questões de segurança.

CHAPTER I – GENERAL INTRODUCTION

I. GENERAL INTRODUCTION

I.1. INCIDENCE OF ADVERSE DRUG REACTIONS AND ITS IMPACT ON PUBLIC HEALTH

The effect of medical innovation over the last century is undeniable. Although pharmacological alternatives have been developed to effectively treat severe diseases, medicines can also cause iatrogenic effects. An adverse event (AE) is defined as any noxious and unintended occurrence that may present during treatment with a drug but which does not necessarily have a causal relationship with this treatment (EUROPEAN MEDICINE AGENCY, 2013). This can be through the use of the drug in its approved conditions but also as a result of misuse (situations where the medicinal product is intentionally and inappropriately used not in accordance with the authorised product information), abuse (persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects) or medication error (EUROPEAN MEDICINE AGENCY, 2013). An adverse drug reaction (ADR) is characterized by the suspicion of a causal association between the exposure to a drug and the occurrence of an adverse event, i.e. judged possible by the reporter or an established causal assessment method (LINDQUIST, 2007).

A survey conducted in noninstitutionalized adults in the United States of America (USA) estimated that more than 90% of the population aged 65 years or older takes at least one medication per week (KAUFMAN *et al.*, 2002). Around 50% of the study' population reported receiving treatment with at least one prescription medicine (KAUFMAN *et al.*, 2002). All medicines can cause unwanted effects, which incidence is expected to increase along with its widespread use. From 1998 through 2005, reported serious adverse drug events in the USA increased 2.6-fold from 34 966 to 89 842, and fatal adverse drug events increased 2.7-fold from 5519 to 15 107 (MOORE, COHEN, and FURBERG, 2007). Notoriously, during the same time period, the overall relative increase of serious AEs reporting was 4 times faster than the growth in total US outpatient prescriptions, which grew in the same period from 2.7 billion to 3.8 billion (MOORE, COHEN, and FURBERG, 2007).

Adverse drug reactions have a significant impact on public health, accounting for considerable morbidity and mortality (GANDHI *et al.*, 2003). Several studies were dedicated to characterize the incidence and the impact of iatrogenic medication disease. For patients receiving treatment in the ambulatory care, the average incidence of AEs is estimated to range from 4 to 91 per 1,000 person-months (THOMSEN *et al.*, 2007). Almost 5% of total

hospital admissions are due to AEs, but in the elderly population this proportion is estimated to be 16.6% (BEIJER and DE BLAEY, 2002). A meta-analysis conducted by Lazarou and colleagues (1998) estimated the overall incidence of serious ADRs as being 6.7% and fatal adverse drug reactions as being 0.32%. Other studies conducted in the hospital setting estimated that 4 to 5% of the inpatients suffered AEs and that fatal AEs have been reported in 0.05 to 0.95% of inpatients. (LEENDERTSE *et al.*, 2010; KANJANARAT *et al.*, 2003; EBBESEN *et al.*, 2001; JUNTTI-PATINEN, and NEUVONEN, 2002).

Additional costs from drug-related morbidity represent a considerable proportion of total costs of health care systems. A study conducted in Sweden estimated the proportions of patients with drug-related morbidity (DRM) and preventable DRM and the cost-of-illness (COI) of DRM in Sweden based on pharmacists 'expert opinion (GYLLENSTEN *et al.*, 2012). It was estimated that the cost-of-illness for treating patients attending healthcare services due to drug-related morbidity would cost 997€ per patient, corresponding to an annual cost of 6.600 million euros to the Swedish healthcare system. In Germany, direct costs of ADRs were estimated as being 3.814€ per patient (STARK, JOHN and LEIDL, 2011).

I.2. BRIEF HISTORICAL BACKGROUND OF DRUG SAFETY AND DRUG SAFETY MONITORING

Pharmacological treatments are perhaps as old as mankind and their iatrogenic events have been reported in literature for many years, in both anecdotal and scientific ways (RÄGGO and SANTOSO, 2008). Drug regulation, in particular regulation for drug safety and for drug safety monitoring, has been impelled upon misfortunate events rather than a rational and knowledge based development (RÄGGO and SANTOSO, 2008).

The USA government established the Bureau of Drugs in order to implement the Biologics Control Act of 1902 (AHMAD, MARKS and GOETSCH, 2006). In early XX century, antitoxin derived from the blood of tetanus-infected retired horses was used to treat diphtheria patients. By 1901, however, in St. Louis, Missouri, 13 children who had been given diphtheria antitoxin died of tetanus (US FOOD AND DRUG ADMINISTRATION, 2009a). The second incident occurred in New Jersey when nine children died from contaminated smallpox vaccine (US FOOD AND DRUG ADMINISTRATION, 2009b). The 1902 Act enacted by the US Congress required annual licensing of manufacturers and distributors and the labelling of all products with the name of the manufacturer (AHMAD, MARKS and GOETSCH, 2006).

In 1906, the original Food and Drugs Act is passed by the US Congress and signed by the president Theodore Roosevelt, prohibiting interstate commerce in misbranded and adulterated foods, drinks and drugs (US FOOD AND DRUG ADMINISTRATION, 2009c). At this time, neither the efficacy nor the pre- and pro-marketing safety of medicines was regulated. It was only in the 1930s that drug safety started be regulated and again due to a disaster.

The S.E. Massengill Co. introduced a sulphanilamide flavoured elixir containing diethylene glycol, an antifreeze. Although the toxic effects of diethylene glycol have been documented 1931 it did not avoid the death of more than 100 people by 1937 (AHMAD, MARKS and GOETSCH, 2006). The Federal Food, Drug and Cosmetic Act was passed by the US Congress in June 1938, requiring that new drugs should be submitted for safety tests before marketing, with the results being submitted to the FDA (AHMAD, MARKS and GOETSCH, 2006). Additionally, this law says that sulphanilamide and other selected dangerous drugs must be administrated under the direction of a qualified person, thus launching the requirement for prescription only (non-narcotic) drugs (US FOOD AND DRUG ADMINISTRATION, 2009c).

Little attention was paid to ADRs until the 1950s when, partially impelled by the developments conducted during the Second World War, pharmaceutical industry experienced an expansion and an increase number of new launched products (AHMAD, MARKS and GOETSCH, 2006). Chloramphenicol was approved in 1949 as a broad-spectrum antibiotic. In 1952 an investigation conducted by FDA revealed that chloramphenicol had caused nearly 180 cases of fatal blood diseases, such as fatal aplastic anaemia (US FOOD AND DRUG ADMINISTRATION, 2009c). In order to gather data to investigate this safety issue, FDA ordered the staff in all 16 district offices to contact every hospital, medical school, and clinic in cities with populations of at least 100.000 to collect information on any cases of blood dyscrasias associated with chloramphenicol (AHMAD, MARKS and GOETSCH, 2006). In few days, 217 cases of chloramphenicol-associated blood dyscrasias were identified (AHMAD, MARKS and GOETSCH, 2006). The result of this experience, coupled with the need to identify serious AEs, as quickly as possible, led the American Medical Association to establish a Committee on Blood Dyscrasias which collected case reports of drug-induced blood related illness (AHMAD, MARKS and GOETSCH, 2006). In 1956, the American Society of Hospital Pharmacists, the American Association of Medical Record Librarians and the American Medical Association piloted the first US drug ADR surveillance program (US FOOD AND DRUG ADMINISTRATION, 2009c). This program began with six hospitals and by 1965 had grown to over 200 teaching hospitals reporting to FDA in a monthly basis.

In the district of Castelo Branco, Portugal, several children died after receiving treatment with an antibiotic during 1957 (CABRITA DA SILVA, SOARES and MARTINS, 2012). This event led the Portuguese authorities to pass the Decree law nº 41448/57, demanding a previous evaluation of new medicines before market access authorization could be granted. At that time, this was a pioneering legislation in Europe (CABRITA DA SILVA, SOARES and MARTINS, 2012). Despite the measures, the Portuguese drug safety monitoring system remained practicly inexistent until the country's admission to the European Economic Community, in 1986.

The so called "thalidomide disaster" is the most remarkable case of iatrogenic worldwide. Thalidomide was a mild hypnotic and sedative which was often used to alleviate morning sickness in pregnant women, going on sale for the first time in Western Germany in 1956 and approved in several countries in the following years (RÄGGO and SANTOSO, 2008). Shortly after thalidomide being approved, it was observed an increase in the frequency of a previously rare birth defect, phocomelia – malformation of limbs (AHMAD, MARKS and GOETSCH, 2006). Dr. William McBride, an Australian physician, reported this adverse drug

reaction for the first time in 1961 (MCBRIDE, 1961). It is estimated that thousands of babies had been exposed to thalidomide during pregnancy. This event led several countries to legislate new regulatory procedures. Although thalidomide was never approved in USA, the US Congress approved the 1962 Kefauver-Harris Amendments to the Food, Drug, and Cosmetic Act of 1938 requiring drug manufacturers to prove to FDA the efficacy and safety of new products before the approval (AHMAD, MARKS and GOETSCH, 2006). Moreover, and for the first time, this law also mandated that pharmaceutical manufacturers must report AEs to FDA for any of their products having a New Drug Application (AHMAD, MARKS and GOETSCH, 2006). The Committee on the Safety of Drugs was established in the United Kingdom (UK) in 1963 and in the following year the Yellow Card Scheme was created allowing physicians to report suspected adverse drug reactions (MEDICINES AND HEALTHCARE PRODUCTS REGULATORY AGENCY, 2013). Other countries like Australia, Canada, Czechoslovakia, Ireland, the Netherlands, New Zealand, Sweden, the United States, and West Germany initiated the systematic collection of cases of suspected adverse drug reactions (EDWARDS et al., 2006).

In 1967, the World Health Assembly of the World Health Organization (WHO) adopted the World Health Assembly (WHA) 20.51 Resolution (Pilot Research Project for International Monitoring of Adverse Reactions to Drugs) which laid the basis for the international system of monitoring ADRs (VENULET and HELLING-BORDA, 2010). The WHO Pilot Research Project for International Drug Monitoring started its operation in Alexandria, Virginia, USA in 1968, with ten countries from Europe, Australasia and North America pooling all reports that had been sent to their national monitoring centres in this WHO project (VENULET and HELLING-BORDA, 2010; EDWARDS et al., 2006). The centre was transferred to the WHO headquarters in Geneva in 1970 before being finally established in Sweden in 1978 as the WHO Uppsala Monitoring Centre (VENULET and HELLING-BORDA, 2010; EDWARDS et al., 2006).

The Portuguese Decree law n° 72/91 adopted the European Union directives, requiring market authorization holders, physicians, pharmacists and other healthcare professionals to report suspected adverse drug reactions to national competent authorities (CABRITA DA SILVA, SOARES and MARTINS, 2012). In 1992, the Normative order n° 107/92 established the Portuguese National Pharmacovigilance System. A year latter the Portuguese national regulatory authority was created under the Decree law n° 353/93 and named *Instituto Nacional da Farmácia e do Medicamento* (INFARMED), now INFARMED - *Autoridade Nacional do Medicamento e Produtos de Saúde* (CABRITA DA SILVA, SOARES and MARTINS, 2012).

This legislation also established the National Pharmacovigilance Centre, which the main purpose was to continue the implementation of the Portuguese National Pharmacovigilance System (CABRITA DA SILVA, SOARES and MARTINS, 2012).

In 1997 it was developed the Computerized Online Medicaid Analysis and Surveillance System (STROM, 2006a). This system used Medicaid billing data to conduct pharmacoepidemiology studies. In 1980 it was developed what today is called the Drug Safety Research Unit in the UK, which is known for being a pioneer in performing Prescription Event Monitoring studies (STROM, 2006a). Both of these novel approaches gave important contributions in the pharmacovigilance field since investigators have gained new tools to generate and investigate research hypothesis on safety issues.

Until the beginning of the 1990s there were five different forms for manufacturers and health care professionals to report medicines' related problems to FDA (AHMAD, MARKS and GOETSCH, 2006). In 1993, it was launched the FDA's MedWatch Adverse Event Reporting Program aiming to facilitate, support and promote the voluntary reporting of suspected adverse drug reactions by health care professionals and consumers (AHMAD, MARKs and GOETSCH, 2006; US FOOD AND DRUG ADMINISTRATION, 2009c).

The European Agency for the Evaluation of Medicinal Products (EMEA) was established in 1995 as well as a new regulatory system which includes procedures for a centralised authorisation (BAHRI, TSINTIS and WALLER, 2007). The agency created the Pharmacovigilance Working Party (PhVWP) of the Committee for Medicinal Products for Human Use (CHMP) to provide recommendations to the CHMP on all matters relating directly or indirectly to pharmacovigilance (EUROPEAN MEDICINES AGENCY, 2005). Upon request of national authorities, the PhVWP could provide recommendations for non-centrally authorised medicines (EUROPEAN MEDICINES AGENCY, 2005). The European Union (EU) pharmacovigilance system was created to supervise the safety of the medicines on the European market. In 2004 EMEA changed its designation to European Medicines Agency (EMA).

The FDA announces the Drug Safety Board, consisting of FDA staff and representatives from the National Institutes of Health and the Veterans Administration (US FOOD AND DRUG ADMINISTRATION, 2009c). This board will advise the Director, Centre for Drug Evaluation and Research (CDER), FDA, on drug safety issues and work with the agency in communicating safety information to health professionals and patients (US FOOD AND DRUG ADMINISTRATION, 2009c).

In 2010, the European Commission reviewed the EU pharmacovigilance system and proposed new legislation (EUROPEAN MEDICINES AGENCY, 2013). This new legislation entered in to force in 2012. Over the years, several EU Member States established schemes for patients directly to report suspected adverse drug reactions to healthcare authorities. The recently implemented EU pharmacovigilance legislation introduced the legal right for European citizens to report suspected adverse drug reaction (EUROPEAN MEDICINES AGENCY, 2013). The Pharmacovigilance Risk Assessment Committee (PRAC) was created and it will meet monthly. The PRAC will advise the CHMP and the Coordination Group for Mutual Recognition and Decentralised Procedures (CMDh) on safety issues associated with medicines authorised for the EU market (EUROPEAN MEDICINES AGENCY, 2013). The PRAC replaced the PhVWP.

Scientific societies dedicated to study the field of drug safety have been created. The International Society on Pharmacoepidemiology (ISPE) was officially launched in 1989 during the 5th International Conference on Pharmacoepidemiology and Risk Management (INTERNATIONAL SOCIETY FOR PHARMACOEPIDEMIOLOGY, 2014). The ISPE is a non-profit international professional membership organization provides a forum to the open exchange of scientific information for the field of pharmacoepidemiology, including pharmacovigilance, drug utilization research, outcomes research, comparative effectiveness research, and therapeutic risk management (INTERNATIONAL SOCIETY FOR PHARMACOEPIDEMIOLOGY, 2014). It counts with members of more than 53 countries. The International Society of Pharmacovigilance (ISOP) aims to foster Pharmacovigilance both scientifically and educationally, and enhance all aspects of the safe and proper use of medicines, in all countries (INTERNATIONAL SOCIETY OF PHARMACOVIGILANCE, 2014). It was created in 1992, initially under the name of European Society of Pharmacovigilance. The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) was created in 2006 by the EU as a project within the European Risk Management Strategy (ERMS) (EUROPEAN NETWORK OF CENTRES FOR PHARMACOEPIDEMIOLOGY AND PHARMACOVIGILANCE, 2014). Its goal is to further strengthen the postauthorisation monitoring of medicinal products in Europe by facilitating the conduct of post-authorisation studies focusing on safety and on benefit-risk (EUROPEAN NETWORK OF CENTRES FOR PHARMACOEPIDEMIOLOGY AND PHARMACOVIGILANCE, 2014). This ENCePP comprises relevant research centres, healthcare databases, electronic registries and existing European networks covering certain rare diseases, therapeutic fields and adverse drug events of interest (EUROPEAN NETWORK OF CENTRES FOR PHARMACOEPIDEMIOLOGY AND PHARMACOVIGILANCE, 2014).

I.3. PHARMACOVIGILANCE

During the clinical development, medicines are tested in a relatively short number of patients. Therefore, a randomized clinical trials (RCT) design are more likely to identify the most frequent and acutely ADRs (MADRE *et al*, 2006 LESKO and MITCHELL, 2012). Once a drug is marketed, more patients will receive treatment and their demographic characteristics are somehow more heterogeneous. Rare and long-latency AEs may arise and subsequently change the initial established benefit/risk profile. Data on drug safety from all available sources need to be collected and managed systematically in order to identify potential drug safety hazards as soon as possible (MADRE *et al*, 2006; STROM, 2012).

The identification of iatrogenic drug disease and the monitoring of its impact on a population perspective led to the development of a discipline of Pharmacoepidemiology - Pharmacovigilance (RAWLINS, 1995). The term "Pharmacovigilance" first appeared in medical literature in 1974 (MOORE, 2013).

Pharmacovigilance is defined as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems (WHO COLLABORATING CENTRE FOR INTERNATIONAL DRUG MONITORING, 2002). It is a multidisciplinary issue involving major disciplines as basic and clinical pharmacology, clinical medicine, toxicology, epidemiology and genetics (AKICI and OKTAY, 2007). The task of Pharmacovigilance is monitoring the safety of medicines and ensuring that the risks of a medicine do not outweigh the benefits, in the interests of public health (EUROPEAN MEDICINES AGENCY, 2013).

Pharmacovigilance has seen significantly developed over the past years (PAL *et al.*, 2011). In developed countries, regulatory authorities have in force rigorous Pharmacovigilance legislations in order to seek greater transparency, accountability and access to information on safety (PAL *et al.*, 2011). In Europe, a new Pharmacovigilance legislation is in force since July 2012 (COMMISSION DIRECTIVE 2010/84/EU, 2010). This new legislation allows EMA to maintain and further develop its tasks, in particular as regards the management of the Union pharmacovigilance database and data-processing network (the 'Eudravigilance database'), the coordination of safety announcements by the Member States and the introduction of the legal right for individual European citizens to report suspected adverse drug reactions.

I.4. METHODS USED IN PHARMACOVIGILANCE

Spontaneously reported suspected ADRs by health care professionals, patients or market authorisation holders (MAHs) are the main source of information on drug safety issues to national pharmacovigilance systems. Since there is a need to quantify and characterize risks to individuals and communities from their medicines and, lately, to minimize their iatrogenic effects, other methodological designs are therefore needed (PAL *et al.*, 2013). The best method to address a specific safety issue depends on a number of variables that should be considered, such as the drug in cause and its therapeutic indication, the population being treated and the AE of interest (EUROPEAN MEDICINES AGENCY, 2005).

The nature of pharmacoepidemiologic study designs which support regulatory decisions on safety issues can be descriptive or analytical (MADRE *et al.*, 2006). Descriptive studies generate hypotheses and describe the occurrence of events related to drug toxicity and/or efficacy (HÄRMARK and VAN GROOTHEEST, 2008). These studies used to obtain the background rate of events and/or establish the prevalence of the use of drugs in specified populations (EUROPEAN MEDICINES AGENCY, 2005). Descriptive studies limit the inference made about causality and include spontaneous case reports, case series, uncontrolled cohorts and registries (MADRE *et al.*, 2006; STROM, 2012). Analytic studies are conducted to test research hypotheses aiming to evaluate the causal association between an observed event and a particular drug or drugs (HÄRMARK and VAN GROOTHEEST, 2008). Analytic studies include a comparator group and there are a variety of designs, such case-control studies, cohort studies and RCTs (MADRE *et al.*, 2006; STROM, 2012). Meta-analyses can be used to combine results from different studies (MADRE *et al.*, 2006; STROM, 2012).

This section aims to provide a summary of the most common pharmacovigilance methods used to study drug safety.

I.4.1. DESCRIPTIVE STUDIES

I.4.1.1. Spontaneous reports

According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) definition, a spontaneous report is an unsolicited communication by a healthcare professional or

consumer to a company, regulatory authority or other organization (e.g. WHO, Regional Centre, Poison Control Centre) that describes one or more adverse drug reactions in a patient who was given one or more medicinal products and that does not derive from a study or any organized data collection scheme (EUROPEAN MEDICINES AGENCY, 2003). Pharmacovigilance centres collect reports of ADRs and evaluate the risk for new safety signals (HÄRMARK and VAN GROOTHEEST, 2008). Marketing authorisation holders also receive reports of their drugs (HÄRMARK and VAN GROOTHEEST, 2008).

Spontaneous reporting systems are the main source of post-marketing information on drug safety (HÄRMARK and VAN GROOTHEEST, 2008). A study conducted to evaluate FDA safety-related drug label changes in 2010 concluded that spontaneous reports contributed to safety-related label changes more than any other evidence source (LESTER *et al.*, 2013). Statistical methods can be applied to spontaneously reported data for signal detection, such as the Proportional Reporting Ratio (PRR), or the method used by the WHO, the Bayesian Confidence Propagation Neural Network (BCPNN). A safety signal can be defined as "information that arises from one or multiple sources (including observations and experiments), which suggests a new potentially causal association, or a new aspect of a known association, between an intervention and an event or set of related events, either adverse or beneficial, which would command regulatory, societal or clinical attention, and is judged to be of sufficient likelihood to justify verifiable and, when necessary, remedial actions" (HAUBEN and ARONSON, 2009). When spontaneously reported cases are the data source, a signal can be defined as a higher than expected relative frequency of a drug-event pair (MADRE *et al.*, DAL PAN, LINDQUIST and GELPERIN, 2012).

Spontaneous report of ADRs is a valuable method to identify rare a serious AEs with an acute onset and occurring with a close temporal relationship between the start of the treatment or following a dosage increment (MADRE *et al.*, 2006; DAL PAN, LINDQUIST and GELPERIN, 2012). Spontaneous reporting systems operate with a relatively low cost, allowing monitoring all drugs in market during their entire life cycles and covering the whole patient population (HÄRMARK and VAN GROOTHEEST, 2008). However, some limitations should be noted.

Adverse events with a long-latency period are less likely to be reported, since only unexpected and severe AEs are prone to be noticed by patients and healthcare professionals. The data accompanying spontaneous reports are frequently incomplete and the rate at which cases are reported is dependent on many factors including the length of time a drug has been on the market, media attention, and the indication for use of the drug

(EUROPEAN MEDICINES AGENCY, 2005). Other limitations of most importance are the underreporting and the selective reporting. A systematic review estimated the median underreporting rate across the included studies was 94% (HAZELL and SHAKIR, 2006). New drugs in market of drugs claiming to be safer are more likely to have their AEs reported (EDWARDS *et al.,* 2006).

Despite the irreplaceable value of spontaneous reporting systems, analytic studies should be conducted to follow-up safety signals generated by this method (EDWARDS *et al.,* 2006; STROM, 2012).

I.4.1.1.1. Targeted Spontaneous Reporting

The Targeted Spontaneous Reporting (TSR) is a variant method from the spontaneous reporting and was developed by the WHO (WORLD HEALTH ORGANIZATION, 2012). With TSR, healthcare professionals managing a group of patients are encouraged to report specific harmful events which are thought to be drug-related (PAL *et al.*, 2013). This method is sustainable and feasible and is being applied as a pharmacovigilance tool in countries with limited human and financial resources (PAL *et al.*, 2013). TSR was piloted for Human Immunodeficiency Virus (HIV) treatment programs in Kenya, Uganda and Vietnam and WHO is planning to use it to collect data on iatrogenic events from tuberculosis treatments (PAL *et al.*, 2013).

I.4.1.2. Case series

A case series are collections of cases, all of whom were subject to the same exposure, whose clinical outcomes are then exposed and evaluated (STROM, 2006; STROM, 2012). Alternatively, case series can be defined as collections of patients suffering the same outcome who will be evaluated in order to identify their previous exposure (STROM, 2006; STROM, 2012). No control group is included in case series. Cases occurring in the same hospital or medical practice constitute often a case series (STROM, 2006; STROM, 2012). This type of study can provide evidence of an association between a drug and an AE, but is more useful as a hypothesis generator methodology (EUROPEAN MEDICINES AGENCY, 2005).

I.4.1.3. Intensive Monitoring

Commonly known as Prescription-Event Monitoring (PEM), an Intensive Monitoring programme is a method of active pharmacovigilance surveillance (EUROPEAN MEDICINES AGENCY, 2005). This methodology was developed in New Zealand (the Intensive Medicines Monitoring Programme) and in the UK (Prescription Event Monitoring) (HÄRMARK and VAN GROOTHEEST, 2008). The design of PEM consists in a non-interventional, observational prospective cohort with the aim of detecting any AE that may present during the treatment with a medicine (SHAKIR, 2007). This type of studies is frequently conducted for new drugs in the early postmarketing phase based on routine clinical practice (PAL *et al.*, 2013). In drug event monitoring, patients might be identified from electronic prescription data or automated health insurance claims (EUROPEAN MEDICINES AGENCY, 2005). Then, a questionnaire is sent to each prescribing physician or patient at specified intervals in order to obtain information on any AE (EUROPEAN MEDICINES AGENCY, 2005). Demographic characteristics of patients, indication for treatment, duration of therapy, dosage, clinical events, and reasons for discontinuation can be included in the questionnaire (EUROPEAN MEDICINES AGENCY, 2005). The physician or patients then return the questionnaire.

This method has a number of strengths (SHAKIR, 2007). It is a non-interventional study which does not interfere with the treatment chosen by the physician, diminishing the risk of selection bias. All the events occurring during the treatment should be reported which can lead to the detection of AEs which could be initially judge as non-related to the drug. Long-term latency ADRs can be explored and the patients' cohort can be followed after the end of the study (SHAKIR, 2007). However, as any other pharmacoepidemiologic study, some limitations should be considered. Not all the questionnaire forms are returned to the sponsor of the study. A PEM depends on physicians reporting, so the underreporting is possible to exist (LAYTON and SHAKIR, 2012). The PEM process developed in the UK by the Drug Safety Research Unit is described in Figure I.1.

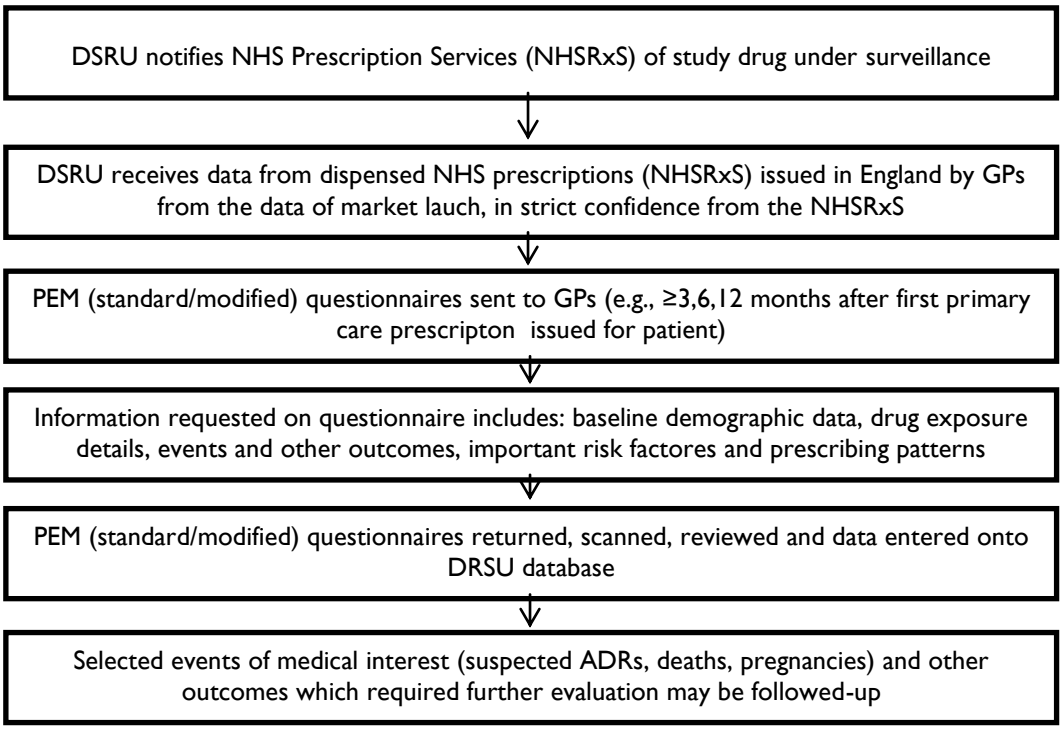


Figure I.1 - The Prescription-Event Monitoring process (LAYTON and SHAKIR, 2012).

Legend: DSRU: Drug Safety Research Unit; NHS: National Health Service; Authority; GP: general practitioner; NHSRxS: NHS Prescription Services; PEM; Prescription-Event Monitoring; ADRs: adverse drug reactions.

I.4.2. ANALYTIC STUDIES

I.4.2.1. Randomized controlled clinical trials

Randomized clinical trials are classified in four phases: phase I, phase II, phase III and phase IV (STANLEY, 2007). RCTs where the pharmacokinetics and pharmacodynamics are initially study are known as phase I RCTs. Phase I RCTs include healthy volunteers. Phase I and phase II RCTs are conducted to establish the initial safety profile, to set the dose range and to collect the first data on the efficacy of the drug (STANLEY, 2007). The initial clinical development of a drug usually includes few hundreds of individuals (ROSENSTOCK *et al.*, 2012). The best evidence on the efficacy and safety of a drug seeking market authorisation or extension of its therapeutic indication is retrieved from RCTs, in particular from Phase III RCTs which include hundreds to thousands of patients (MADRE *et al.*, 2006; LESKO and MITCHELL, 2012). The time length depends on the relative incidence of the chosen endpoint and the simple size is estimated based on the power required to demonstrate a statistical

different between groups on a clinical effect (MADRE *et al.*, 2006; LESKO and MITCHELL, 2012). The phase IV RCTs, conducted after a medicine is marketed, aim to provide additional details about the medicine's efficacy or safety profile (AMGEN, 2012). The detection and more rigorous assessment of previously unknown or inadequately quantified AEs are an important role of phase IV RCTs (STROM *et al.*, 2008). A particular type of clinical trial useful to conduct postmarketing safety profile assessments is the Large Simple Trial (LST). Large Simple Trials are considered the best solution when it is not possible to completely contro confounding by means other than randomization (LESKO and MITCHELL, 2012). This type of RCT has been used to study the risk of adverse drug effects when observational designs may be judged inadequate (LESKO and MITCHELL, 2012; LESKO and MITCHELL, 1995).

The most frequent and acute drug-related AEs can be detected during the pre-market clinical development (MADRE *et al.*, 2006; LESKO and MITCHELL, 2012). Most of what is known about the safety profile of a drug comes from harmful effects reported during RCTs. Nonetheless, it is highly probable that serious unexpected suspected ADRs may occur after a drug being introduced in market. The total patient size of Phase III RCTs commonly rounds the few thousands (MADRE *et al.*, 2006; LESKO and MITCHELL, 2012). Clinical trials are not designed to evaluate the risk for rare and/or long-term latency AEs. Other limitation of RCTs is the inclusion/exclusion criteria for patients (MADRE *et al.*, 2006; LESKO and MITCHELL, 2012). Patients included in RCTs are treated in well-defined indication and may receive a limited number of concomitant drugs (MADRE *et al.*, 2006; LESKO and MITCHELL, 2012). Additionally, individuals of particular groups such as elderly, children or pregnant women are usually underrepresented or excluded (HÄRMARK and VAN GROOTHEEST, 2008). When safety issues arise from RCTs they usually occur unexpectedly and most of the times they are not prespecified outcomes (BOMBARDIER *et al.*, 2000).

Since RTCs are mainly conducted to demonstrate clinical efficacy during clinical development they tend to be unnecessary after a market authorization to be granted (STROM, 2006; STROM, 2012). The exception is when therapeutic indication extensions are required. Ethical issues restrain RCTs to be conducted in order to evaluate safety issues. However, in some situations such studies can be conducted in order to analyze the serious risks arising from medicines, in particular if such safety concerns could be adequately expressed as safety endpoints (US FOOD AND DRUG ADMINISTRATION, 2011). According to FDA guidance, examples include RCTs designed to (US FOOD AND DRUG ADMINISTRATION, 2011):

- Evaluate the occurrence of asthma exacerbations associated with an irritative component of inhalation treatments for asthma in a RCT, where the increased risk of drug-related exacerbation has the potential to offset the effectiveness of the inhaled drug;
- Determine the incidence of myocardial infarction in patients treated with the approved drug in a follow-on trial after approval, using the original randomized population;
- Evaluate differences in safety outcomes between patients withdrawn from treatment after some period of treatment and patients who remain on the treatment (randomized withdrawal trial);
- Evaluate the potential for QT interval prolongation in a thorough QT interval RCT;
- Measure growth and neurocognitive function in pediatric patients treated chronically with the drug*;
- Evaluate safety in a particular racial or ethnic group or vulnerable population such as the immunocompromised*;
- Evaluate the safety of the drug in pregnant women*;
- Evaluate drug toxicity in patients with hepatic or renal impairment*;
- Evaluate long-term safety of cell and gene therapy products depending on the type of vector used and the inherent risk of integration;
- Evaluate the safety of a drug in patients with HIV-1 co-infected with hepatitis C or B*

* - *Patients are treated with the drug at a dose and schedule specified in the RCT protocol.*

Due to ethical and design limitations of RCTs, observational study designs are main data sources supporting pharmacovigilance activities.

I.4.2.2. Observational studies

I.4.2.2.1. Case-control studies

A study authored by Janet Lane-Claypon in 1926 and published by the British Ministry of Health entitled "A further report on cancer of the breast: reports on public health and medical subjects." is considered the first case-control study (PANETH, SUSSER and SUSSER, 2002). In case-control studies, cases with a disease (or event) are compared with controls

without the disease looking for differences in previous exposures (Figure I.2) (STROM, 2006; ROSENBERG, COOGAN and PALMER, 2012). The prevalence of exposure among the controls should represent the prevalence of the exposure in the source population (EUROPEAN MEDICINES AGENCY, 2005).

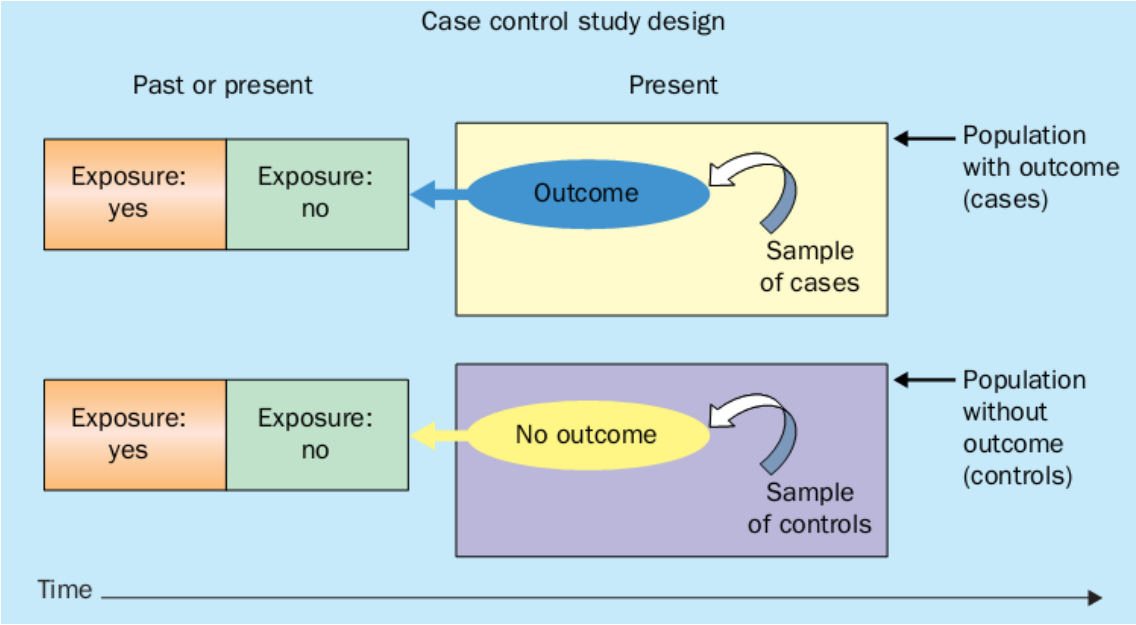


Figure I.2 - Schematic diagram of a case-control study design (SCHULZ and GRIMES, 2002).

Case-control studies are useful when it is aimed to study multiple causes of a disease since the same cases and controls can be used to examine any number of exposures as potential risk factors (STROM, 2006; ROSENBERG, COOGAN and PALMER, 2012). This design is often used to investigate the risk for developing rare AEs or the cause of a disease with a long latency period, since conducting a cohort study with the same objective would be less efficient in terms of time, money and efforts (SCHULZ and GRIMES, 2002). Information on individuals' exposures is generally obtained in a retrospective fashion. Patients are commonly recruited from databases containing their medical records, but data can be collected specifically for the study, by administering questionnaires or conducting interviews (EUROPEAN MEDICINES AGENCY, 2005). This retrospective data collection procedure on the exposure has the risk of a poor validation (STROM, 2006; ROSENBERG, COOGAN and PALMER, 2012). Moreover, controls selection is difficult and its inappropriate sampling can introduce bias in the study (STROM, 2006; ROSENBERG, COOGAN and PALMER, 2012).

Incidence rates cannot be estimated from case-control studies (SCHULZ and GRIMES, 2002). Instead, the odds ratio (OR) should be used to compare the different proportion of

exposed individuals among cases and controls groups (SCHULZ and GRIMES, 2002; EUROPEAN MEDICINES AGENCY, 2005). The OR is a good estimate of the relative risk (RR) when rare events are being studied (SCHULZ and GRIMES, 2002).

I.4.2.2.2. Cohort studies

Wade Hampton Frost, an epidemiologist, was the first to use the word "cohort" in his publication assessing the age-specified mortality rates and tuberculosis, in 1935 (FROST, 1935; SONG and CHUNG, 2010). In cohort studies, a population-at-risk for a disease or an event is followed over time for the occurrence of such disease or event (EUROPEAN MEDICINES AGENCY, 2005). Cohort studies can be prospective or retrospective, since investigators can go forward in time or go back in time to select the cohort (Figure I.3) (GRIMES and SCHULZ, 2002).

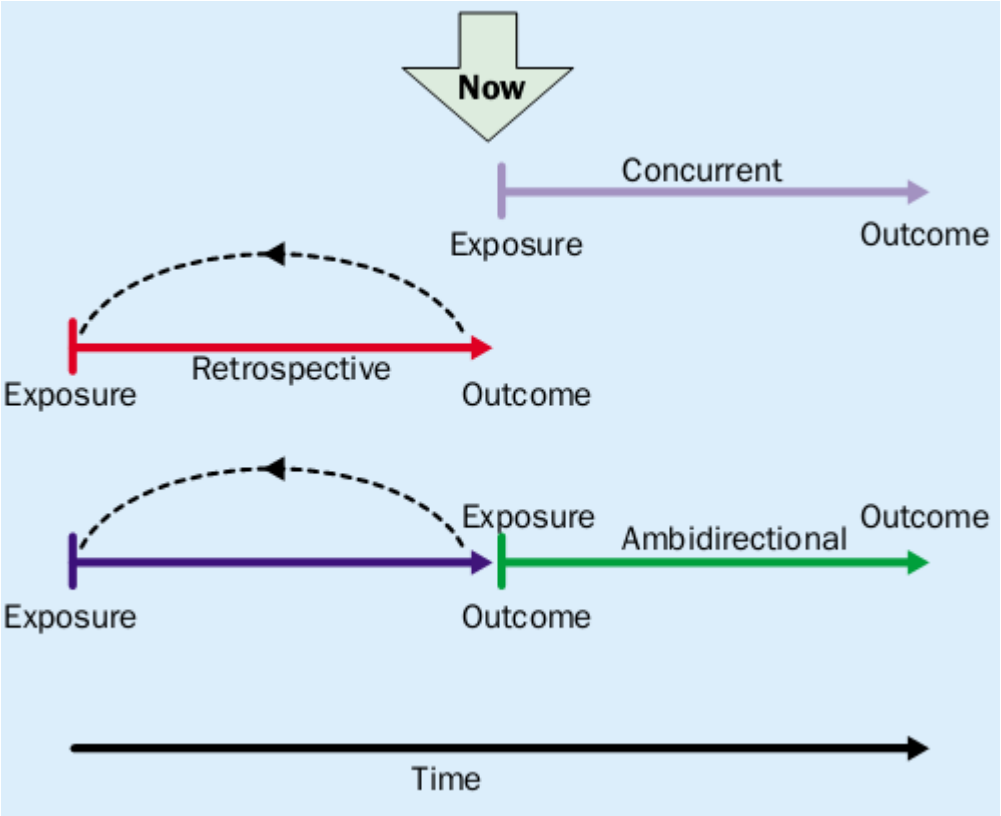


Figure I.3 - Schematic diagram of cohort study design possibilities: concurrent (prospective), retrospective and ambidirectional (GRIMES and SCHULZ, 2002).

A bidirectional design can also exist when data is collected in both directions (GRIMES and SCHULZ, 2002). Typically, two or more groups of patients are followed from exposure to outcome (GRIMES and SCHULZ, 2002). Usually, cohort studies are used to compare a group of exposed patients to a group of unexposed or to compare groups under different exposures (STROM, 2006; STROM, 2012). If the groups have different frequencies in outcomes, then an association can be suggested (GRIMES and SCHULZ, 2002).

The basic difference between cohort and case-control studies is the way that the patients are recruited into the study (GRIMES and SCHULZ, 2002). Into a cohort study, patients are recruited based on presence of absence of an exposure and are then followed over time to study their disease course (GRIMES and SCHULZ, 2002). Patients are recruited into a case-control study based on presence of absence of an outcome (e.g: AE or disease), and their previous exposures are then evaluated (GRIMES and SCHULZ, 2002).

Cohort studies offer several advantages. They are the best design to document the natural history of a disease or the incidence of an AE (GRIMES and SCHULZ, 2002). Multiple outcomes can be investigated using the same data source (EUROPEAN MEDICINES AGENCY, 2005). However, this can arise methodological issues since the sample size may not be large enough to study rare events. Cohort studies are useful to estimate incidence rates and relative risks (RRs) and they can reduce the risk of survival bias when compared with case-control studies (GRIMES and SCHULZ, 2002). As examples of limitations of cohort studies, one can be pointed out are the risk for selection bias, the difficult of studying the risk for rare and long latency events and the lost for follow-up of patients (STROM, 2006; STROM, 2012; GRIMES and SCHULZ, 2002).

I.4.2.2.3. Nested case-control studies

The nested case-control design differs from the traditional case-control design in that it is "nested" in a well-defined cohort, for which information on all members can be obtained (ESSEBAG *et al.*, 2003). The nested case-control design was introduced by Mantel in 1973. There are four crucial steps in the design of nested case-control studies: define the cohort's time axis; select all the cases (all subjects with the outcome of interest); form all risk sets corresponding to the cases, and; randomly select one or more controls from each risk set (SUISSA, 2006; SCHNEEWEISS and SUISSA, 2012). The risk set consist in all noncases (considered to be at risk of becoming cases) present in the cohort at the time the case becomes a case

(ESSEBAG *et al.*, 2003). The most appropriate method to select controls is random selection, without replacement, of noncases presented at the risk set of each case (ESSEBAG *et al.*, 2003). Selection of controls only from noncases or not use subjects more than once as controls can introduce bias in the risk estimation (SUISSA, 2006; SCHNEEWEISS and SUISSA, 2012). A case-control study design is illustrated in Figure I.4 (ESSEBAG *et al.*, 2003).

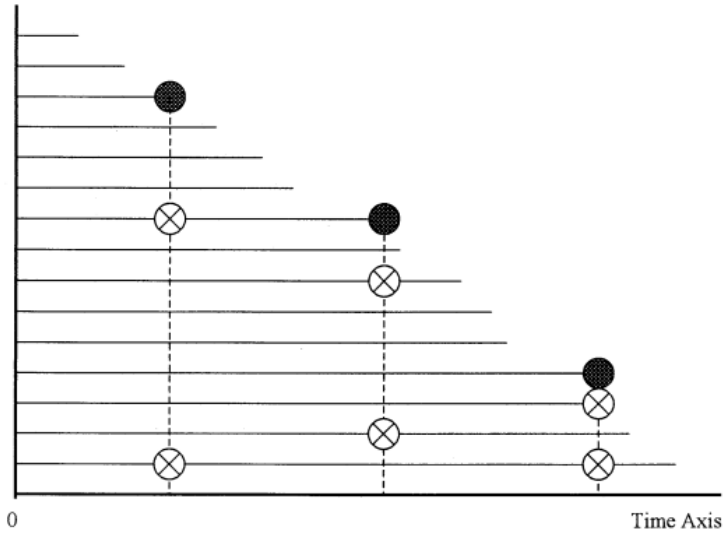


Figure I.4 - The design of a nested case-control study (ESSEBAG *et al.*, 2003). In this example, there are 2 controls (white circles with a X) for every case (black circles). Follow-up is represented by horizontal black lines, beginning at the zero-time. One should be notice that a future case may be selected as a control for a prior case, and that a given subject may be selected as a control for 2 different cases.

Nested case-control studies are used to conduct internal comparisons (within the cohort) between exposures to different drugs (SUISSA, 2006; SCHNEEWEISS and SUISSA, 2012). This design can also be used to compare the rates of AEs within a cohort with those occurring in the general population, with proper adjust for variables such sex or age (SUISSA, 2006; SCHNEEWEISS and SUISSA, 2012).

I.4.2.2.4. Case-cohort studies

The design of a case-cohort study was proposed by Prentice in 1986 (PRENTICE, 1986; ESSEBAG *et al.*, 2003). A case-cohort study begins with the definition of a cohort time axis, followed by the selection of all cases, like a nested case-control design (Figure I.5) (SUISSA, 2006; SCHNEEWEISS and SUISSA, 2012). The difference is the controls' selection process. In a case-cohort study, a random sample of a predetermined size, usually called subcohort, is

selected from the cohort (SUISSA, 2006; SCHNEEWEISS and SUISSA, 2012). For every case, a risk set is established (as in the nested case-control study) of all noncases at risk at the time the case becomes a case (ESSEBAG *et al.*, 2003). All members of the predefined subcohort present in a case's risk set are used as controls for the case (as opposed to the random selection of *X* controls from each case's risk set in the nested case-control study) (ESSEBAG *et al.*, 2003). It is possible that a future case included in the subcohort serves as a control for all cases occurring before the future case becomes a case (ESSEBAG *et al.*, 2003).

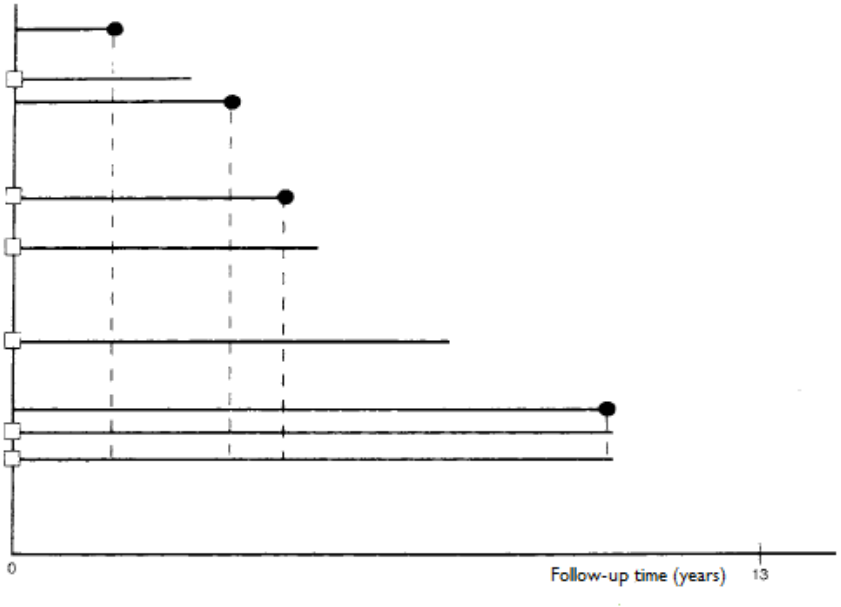


Figure I.5 - The design of a case-cohort study (SUISSA, 2006). In this example, there are 4 cases (black circles). Follow-up is represented by horizontal black lines, beginning at the zero-time.

I.4.2.2.5. Cross-sectional studies

A cross-sectional study examines exposure(s) and outcome(s) in a population at one point in time; they have no time sense. These studies are conducted to gather data for surveys or for ecological analyses (EUROPEAN MEDICINES AGENCY, 2005). No temporal relationship between an exposure and an outcome can be directly addressed (EUROPEAN MEDICINES AGENCY, 2005). These studies can estimate the prevalence of an event at a time in a population.

I.4.2.3. Registry studies

According to the Agency for Healthcare Research and Quality (AHRQ) definition "a patient registry is an organized system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition, or exposure, and that serves one or more predetermined scientific, clinical, or policy purposes." (GLIKLICH and DREYER, 2010). Registries conducted to collect data on diseases, such blood dyscrasias, severe cutaneous reactions, or congenital malformations can be used to investigate previous drug exposures or other factors associated with the clinical condition (EUROPEAN MEDICINES AGENCY, 2005). This method can be used to perform case-control studies since drug exposure from cases identified in the registry can be compared with drug exposure from controls which can be selected from either patients with other condition from the registry or patients outside the registry (EUROPEAN MEDICINES AGENCY, 2005). Registries can also be conducted to collect data exposure to drugs of interest, called exposure registries (EUROPEAN MEDICINES AGENCY, 2005). Exposure registries focus on patients treated with a particular drug, studying the effects of the selected therapeutic in a population of interest, such pregnant women (GLIKLICH and DREYER, 2010). In both types of registries, forms such as a questionnaire or an AEs' case report form can be used to collect the information from providers or patients in a prospective way (GLIKLICH and DREYER, 2010). Cohort studies can be conducted since registries allow patients to be followed over time (EUROPEAN MEDICINES AGENCY, 2005). Despite no control group is included in this methodology, registries allow studying the incidence of AEs and, in the presence of new evidence, they can be used for signal generation (EUROPEAN MEDICINES AGENCY, 2005).

I.4.2.4. Studies conducted based on automated databases

Once a hypothesis for a safety signal is generated it is necessary to test the hypothesis, most of times by conducting longitudinal studies, such cohorts or case-controls. Since rare and/or long-term latency serious suspected adverse drug reactions are those of most concern, postmarketing studies usually included 10000 exposed persons in a cohort study or enrol diseased patients from a population of equivalent size for a case-control study (STROM, 2006b; STROM, 2012a). It may not be feasible and cost-effective to conduct studies requiring such sample sizes and time length by collecting original primary data. For all these

reasons, the use of computerised automated databases as data sources for pharmacoepidemiology studies has grown in the past decades (STROM, 2006b; STROM, 2012a). Automated databases have been used for pharmacoepidemiology research in North America since 1980s and they were initially created for administrative purposes (STROM, 2006b; STROM, 2012a).

There are two main types of automated databases (EUROPEAN MEDICINES AGENCY, 2013). Those that contain comprehensive medical information, which include prescriptions, diagnosis, referrals and discharge reports, such the Clinical Practice Research Datalink (CPRD). The other type of databases are those mainly created for administrative purposes, which require a record-linkage between pharmacy claims and medical claims databases, such the PHARMO system in The Netherlands or the Medicaid in the USA (EUROPEAN MEDICINES AGENCY, 2013). These databases may include data on millions of patients. However, they may not have the detailed information on some variables that are valuable more accurate research, such as validated diagnostic information or laboratory data (EUROPEAN MEDICINES AGENCY, 2013). Guidelines were created to assist investigators in the selection and use of data resources for an observational study in pharmacoepidemiology by highlighting potential limitations and recommending tested procedures (HALL *et al.*, 2012).

1.4.3. STUDIES' METHODOLOGICAL QUALITY ASSESSMENT

The critical appraisal of the methodological quality of studies included in systematic reviews or meta-analysis is an essential feature (MOJA *et al.*, 2005). The methodological quality can be considered a multidimensional concept, which could relate to the design, conduct, and analysis of a study, its clinical relevance, or quality of reporting (JÜNI, ALTMAN and EGGER, 2001). Defects in the methodological quality of studies may bias their results, and hence the results of meta-analyses where they were included (WOOD et al., 2008). Kjaergard and colleagues (2001) conducted a study to explore whether reported methodologic quality affects estimated intervention effects in RCTs and contributes to discrepancies between the results of large sized RCTs and small sized RCTs in meta-analyses. The authors found that, compared with large RCTs, estimates of intervention benefits were exaggerated in small RCTs with inadequate allocation sequence generation, inadequate allocation concealment, and no double blinding (KJAERGARD, VILLUMSEN and GLUUD, 2001). Effect size estimates from large RCTs did not differ significantly from small RCTs with adequate generation of the allocation sequence, adequate allocation concealment, or adequate double blinding (KJAERGARD, VILLUMSEN and GLUUD, 2001). Moher and colleagues (1998) replicated 11 meta-analyses in order to explore the effects of quality on the quantitative results. The authors concluded that the interpretation of the benefit of an intervention from the results of a meta-analysis including low methodological quality studies can be altered (MOHER *et al.*, 1998). However, other studies did not find a correlation between studies' methodological quality and differences in estimated effect sizes (WOOD et al., 2008).

There are several instruments and checklists to assess the methodological quality of RCTs and non-randomized studies. The assessment of randomization process, concealment of allocation process, blinding of participants and outcomes assessment, patients' withdrawal or selective outcome reporting are some of domains assessed by investigators regarding RCTs quality (JÜNI *et al.*, 1999). Nevertheless, the number of items considered for evaluation from one instrument to another can vary significantly (JÜNI *et al.*, 1999). The recommendations from the Cochrane Collaboration, or the scales of Jadad and colleagues (1996), Destky and colleagues (1992) or Chalmers and colleagues (1981) are examples of instruments frequently used to critically appraisal RCTs methodological quality. Deeks and colleagues (2003) conducted a review of methods and related evidence for evaluating bias in non-randomized studies. They identified 194 different instruments and scales used to assess

the methodological quality of non-randomized studies (DEEKS *et al.*, 2003). The allocation process, the comparability of groups, and the adjustment of data for sociodemographic characteristics of patients, also called case-mix adjustment, are domains taken into consideration when assessing the methodological quality of non-randomized studies (DEEKS *et al.*, 2003). The Downs and Black instrument (1998) and the Newcastle-Ottawa Scale (WELLS *et al.*, 2014) are examples of instruments frequently used to assess the methodological quality of non-randomized studies.

I.5. META-ANALYSIS

Meta-analysis is defined as "the statistical analysis of a collection of analytic results for the purpose of integrating the finding" (BERLIN, CEPEDA and KIM, 2012). This involves the statistical combination of summary statistics from various studies but this technique can also combine raw data (EUROPEAN MEDICINES AGENCY, 2001). One should make a distinction between meta-analysis and systematic review. The term 'meta-analysis' should be restricted to the process of statistical synthesis. A systematic review comprises "the application of strategies that limit bias in the assembly and critical appraisal of all relevant studies on a specific topic" (CHALMERS and ALTMAN, 1995). A meta-analysis may be, but not necessarily, part of systematic review process.

There are a number of reasons to conduct a meta-analysis (EUROPEAN MEDICINES AGENCY, 2001):

1) To provide a more precise estimate of the overall treatment effects;
2) To evaluate whether overall positive results are also seen in pre-specified subgroups of patients;
3) To evaluate an additional efficacy outcome that requires more power than the individual trials can provide;
4) To evaluate safety in a subgroup of patients, or a rare AE in all patients;
5) To improve the estimation of the dose-response relationship;
6) To evaluate apparently conflicting study results.

I.5.1. THE EVOLUTION OF RESEARCH SYNTHESIS AND META-ANALYSIS

An historical perspective on the evolution of research synthesis has been presented by Keith O'Rourke (2007) in a paper published in 2007. The study of Karl Pearson was one of the firsts to combine observations from different clinical studies (PEARSON, 1904). Published in 1904, this study was conducted to evaluate the effectiveness of typhoid vaccine, by comparing the infection and mortality rates among soldiers who have been inoculated with those who have not. Besides medical sciences, research synthesis was conducted addressing issues in other scientific fields, like astronomy and zoology (SUTTON, and HIGGINS, 2008).

Despite initial research synthesis has been developed more than one century ago, their acceptance and frequent application took a while. Combine data from different studies

and take conclusion on an investigational issue was frequently done throw narrative reviews (BORENSTEIN *et al.*, 2009). Studies addressing a research question were reviewed by an expert in the field, who summarized the findings and discussed the results, and then reached a conclusion. However, since a narrative review is a subjective procedure by nature, this technique has been abandoned by researches. Systematic reviews and meta-analyses started to gain relevance due to the need for medical research and clinical practice to be based on the most robust scientific evidence (SUTTON, and HIGGINS, 2008). These techniques use a set of rules to search for studies, and then to determine which studies will be included or excluded from the analysis (BORENSTEIN *et al.*, 2009).

The increasing number of research reports led investigators to develop methods to synthesize results from different studies (O'ROURKE, 2007). One of the first quantitative syntheses of identical studies concerning a common research issue was published in 1940 by JG Pratt and colleagues, which evaluated the more than 100 reports on extrasensory experiments (O'ROURKE, 2007). The term "meta-analysis" was coined by Gene V. Glass in 1976 (1976), which he referred to "the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings". An editorial was anonymously published in *The Lancet* journal in 1980 reported a meta-analysis assessing whether aspirin could reduce the risk for myocardial infarction (ANONYMOUS, 1980; O'ROURKE, 2007). This meta-analysis was conducted by Archibald Cochrane and Peter Elwood due to doubts surrounding the beneficial effect of aspirin in cardioprotection (ELWOOD *et al.,* 1974; O'ROURKE, 2007). Many meta-analyses have been published ever since, as well as books and papers on meta-analysis' methodology (O'ROURKE, 2007).

Archibald Cochrane drew attention to the lack of organized critical summary of clinical evidence, by specialty, which could be updated periodically in order to support physicians to perform clinical judgments (VOLMINK *et al.,* 2004). The UK's National Perinatal Epidemiology Unit is a multidisciplinary research unit which was established at the University of Oxford in 1978. This working group conducted "methodologically rigorous research to provide evidence to improve the care provided to women and their families during pregnancy, childbirth, the newborn period and early childhood as well as promoting the effective use of resources by perinatal health services." The investigators systematically reviewed RCTs in perinatal medicine, using meta-analysis when appropriate and possible, which resulted on a two-volume book "Effective Care in Pregnancy and Childbirth" and in a companion volume "Effective Care of the Newborn Infant" (STARR *et al.,* 2009). The knowledge produced by this international collaboration led Michael Peckham, first Director

of Research & Development in the British National Health Service, to approve funding for 'a Cochrane Centre' "to facilitate the preparation of systematic reviews of RCTs of healthcare" (THE COCHRANE COLLABORATION, 2013a). In the following year, the Cochrane Collaboration was inaugurated in Oxford, England, as well as the firsts Cochrane Review Groups, aiming to collate and synthesize high-quality evidence on the effects of healthcare interventions which results could be consulted by a worldwide multi-disciplinary audience (SUTTON, and HIGGINS, 2008). The contribution of the Cochrane Collaboration was recognized as leading to important methodological advances in the systematic reviews and meta-analyses field (GRIMSHAW, 2004). Additionally, the Cochrane Reviews were considered being of great quality (GRIMSHAW, 2004). In 2013, more than 31,000 dedicated people from over 120 countries integrated the Cochrane Collaboration international network, with more than 5000 Cochrane Reviews published online in the Cochrane Database of Systematic Reviews (THE COCHRANE COLLABORATION, 2013B).

I.5.2. CLINICAL RELEVANCE OF CONDUCTING A META-ANALYSIS

Meta-analysis has been widely explored by investigators as a methodology to conduct clinical research (PATSOPOULOS, ANALATOS and IOANNIDIS, 2005). Investigators may have several interests to conduct a meta-analysis aiming to clarify a research question which may not be properly addressed with other study designs (BERLIN, CEPEDA and KIM, 2012). In several occasions, the study of rare AEs relies on pooled analysis. Meta-analysis may be a valuable tool when there is the need to explore inconsistencies across studies previously conducted, to evaluate subgroups of patients in whom an intervention may be more or less effective or to compare the efficacy and/or safety of several interventions.

By applying statistical methods and pooling an estimate of the effect size, meta-analysis allows discussing the magnitude of the effect between the intervention being evaluated and the selected comparator (BORENSTEIN *et al.*, 2009). Narrative reviews do not provide mechanisms to synthesise data and present a subjective evaluation of the selected studies (BORENSTEIN *et al.*, 2009).

I.5.3. A PROTOCOL TO CONDUCT A META-ANALYSIS

A meta-analysis should be conducted according to a prespecified protocol and the steps involved in the process should be clarified before starting the work (MOHER *et al.*,

1999; MOHER *et al.*, 2009; STROUP *et al.*, 2000; EUROPEAN MEDICINES AGENCY, 2001). Guidelines have been developed to improve the quality of the reporting of meta-analyses. Investigators can find recommendations to report meta-analysis of randomised trials, like The Quality of Reporting of Meta-analyses (QUOROM) statement, lately updated by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) recommendations (MOHER *et al.*, 1999; MOHER *et al.*, 2009). The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines have been developed to help reporting meta-analysis of observational studies (STROUP *et al.*, 2000). These guidelines were also developed to help reviewers, editors, and readers to interpret meta-analyses. Defining a protocol based on such recommendations allows the replication of the meta-analysis. Generally, several points should be considered:

1) Define precisely the objective of the meta-analysis and state the investigational hypothesis.
2) Perform a literature search:

   The authors should report the search strategy with the keywords and the index terms, specify which databases were searched, report if hand searching or contact with authors were employed or not, if literature other than English was consulted, or if unpublished material was used.
3) Establish the inclusion/exclusion criteria:

   Based on the initially established objective, the author should set of rules for including and excluding studies from the meta-analysis. These criteria are usually based on study design (e.g.: only RCTs, only observational studies or include both type), study populations (e.g.: elderly, pregnant, young patients, or establish age groups), different treatment dosages, or different studies' duration.
4) Describe the data collection process:

   Describe the methodology used to extract data from studies' reports (e.g.: extraction by investigators in an independently fashion, extraction in duplicate) and the process used to confirm the data extracted.
5) Statistical methods employed:

   The authors should describe the statistical methodology employed to conduct the meta-analysis. Which effect size was used (e.g.: OR, RR, Risk difference [RD]); which meta-analytic model was used (e.g.: fixed or random effects models) and the justification of whether the chosen models account for predictors of study results; describe if the association between two variables was

evaluated by means of a meta-regression (e.g: dose-response effect, age-severity of event); describe if cumulative meta-analysis was used to evaluate who results perform over time. The authors should also state which methods were used to evaluate between-study heterogeneity and the publication bias.

6) Evaluate the consistency of the results:

   It is usual to describe a sensitivity analysis which allows evaluating the consistency of the results. For example, integrate data according different study designs, study durations, according different treatment doses or subpopulations.

7) Formulate conclusions and recommendations according the results.

I.5.4. EFFECT SIZE MEASURES

The effect size is the term used to refer to OR, RR or RD, which are common in meta-analyses that deal with medical interventions (BORENSTEIN *et al.*, 2009). Different effect size measures are used depending on when there is a need to quantify a relationship between two variables or a difference between two groups must be calculated (BORENSTEIN *et al.*, 2009).

According to Borenstein and colleagues (2009), there are some considerations that should drive the choice of an effect size. First, the effect size from different studies should be comparable to one another since they measure the same thing. Secondly, the estimate of the effect size should be computable from the information that is reported in the publish literature, as an article, and should not require the re-analysis of the raw data. Third, an effect size should have good technical properties, like knowing its sampling distribution in order to allow the estimation of its variance and confidence intervals.

Borenstein and colleagues (2009) also refer that most of the times the selected effect size is based on the kind of data that was reported in the primary studies. Table I.1 describes the more common effect sizes and the correspondent study designs. If the summary data reported in the primary studies are based on a binary outcome, such events and non-events in two groups, then the appropriate effect size to select it will be the relative risk (RR), the OR or the RD. These effect size measures can be calculated from 2 x 2 tables. If, instead, the summary data reported in the primary studies are based on means and standard deviations in two groups, it will be more appropriate to select the raw difference in means or the standardized mean difference. A correlation coefficient should be selected when a correlation between two variables is reported in primary data. There is also the option of

compute time-to-event outcomes, also called survival analysis (HIGGINS and GREEN, 2011). Time-to-event data arise when the interest is focused on the time elapsing before an event is experienced. The most appropriate way of summarizing time-to-event data is to use methods of survival analysis and express the intervention effect as a hazard ratio (HR) (HIGGINS and GREEN, 2011). The notion of risk and hazard is similar, but is subtly different in that it measures instantaneous risk and may change continuously (e.g.: the hazard of death changes as someone crosses a busy road) (HIGGINS and GREEN, 2011). Hazard ratio is interpreted similarly to RR, as it describes how many times more (or less) likely a participant is to suffer the event at a particular point in time if they receive the experimental rather than the control intervention. Hazard ratios are computed for each study and meta-analysis is used to integrate data from all studies (BORENSTEIN *et al.*, 2009).

Table I.1 - Most common effect size measures used in meta-analysis and their correspondent study designs (adapted from BORENSTEIN *et al.*, 2009).

Effect sizes based on means (continuous data)
Raw (unstandardized) mean difference (D)
Based on studies with independent groups
Based on studies with matched groups or pre-post designs
Standardized mean difference (d or g)
Based on studies with independent groups
Based on studies with matched groups or pre-post designs
Effect sizes based on binary data
Relative risk (RR)
Based on studies with independent groups
Odds ratio (OR)
Based on studies with independent groups
Risk difference (RD)
Based on studies with independent groups
Effect size based on correlational data
Correlation (r)
Based on studies with one group

I.5.5. STATISTICAL ANALYSES

I.5.5.1. Statistical models

Two models can be used to assess the way in which the variability of the results between the studies included in the meta-analysis is treated: the fixed effects model and the random effects model (PANESAR, SIOW and ATHANASIOU, 2010).

I.5.5.1.1. Fixed effects meta-analysis

Under the fixed effects model it is assumed that all studies in the meta-analysis share a common (true) effect size and that all differences in observed effects are due to sampling error (BORENSTEIN *et al.*, 2009). In other words, if a meta-analysis of ORs is being conducted, it is assumed that all studies estimate the same OR (PANESAR, SIOW and ATHANASIOU, 2010). If each study had an infinite sample size the sampling error would be zero and the observed effect for each study would be the same as the true effect (BORENSTEIN *et al.*, 2009). However, since the simple size of each study is not infinite, there is sampling error and the observed effect in the study is not the same as the true effect.

In the context of a fixed effects meta-analysis, each is assumed to be a random representative conducted on a homogeneous population of patients, assuming that each study is identical to one another (PANESAR, SIOW and ATHANASIOU, 2010). Thus, this model assumes that there is no statistical heterogeneity among the studies and the summary measure is a simple weighted average and can be interpreted as an estimate of a single population outcome measure (PANESAR, SIOW and ATHANASIOU, 2010).

In a fixed effects meta-analysis, the methods used to analyse binary outcome data are: the inverse variance-based method, the Mantel-Haenszel method, and the Peto's method. The inverse variance-based method is so called because the weight attributed to each study is calculated as the inverse of the variance of the effect estimated, or the inverse of the square of its standard error (BORENSTEIN *et al.*, 2009). Larger studies with smaller standard errors are given more weight than small sized studies with larger standard errors (PANESAR, SIOW and ATHANASIOU, 2010). The weighted average (estimated effect size) calculation based on the fixed effects model is given by:

$$M = \frac{\sum_{i=1}^{k} WiYi}{\sum_{i=1}^{k} Wi}$$

where W_i is

$$Wi = \frac{1}{V_{Yi}}$$

V_{Yi} is the within-study variance for study *i*.

For most purposes the inverse variance-based method is appropriate, but when studies have small sample sizes, or when events are rare, other methods can have better statistical properties (BORENSTEIN *et al.*, 2009). When studies have large sample sizes, the variance estimation is close to the true variance of that study, as assumed by the inverse variance-based method. However, the variance may not be well estimated when studies are small or the events are rare (BORENSTEIN *et al.*, 2009). The Mantel-Haenszel method uses a different weighting scheme based on which effect size measure is being used (e.g: RR, OR or RD) and not requiring variance to be estimated (BORENSTEIN *et al.*, 2009). The Mantel-Haenszel method has better statistical properties when there are few events and has become the default method for the fixed effects analysis (PANESAR, SIOW and ATHANASIOU, 2010). The Mantel-Haenszel method was developed to combine OR across 2 x 2 tables and it has since been extended to combine RR or RD across 2 x 2 tables (BORENSTEIN *et al.*, 2009).

The Peto's method, also called the one-step method, can only be used to pool ORs and is a variant of the inverse variance-based method (BORENSTEIN *et al.*, 2009). This method uses an approximate method to estimate the log OR and uses different weights (PANESAR, SIOW and ATHANASIOU, 2010).

The inverse variance-based formula does not work well when one or more cells in the 2 x 2 table have a value of zero (BORENSTEIN *et al.*, 2009). The approach is to add the value of 0.5 to all four cells. Both the Mantel-Haenszel and Peto's methods are able to work with zero values and no adjustments are needed (BORENSTEIN *et al.*, 2009). However, in the Peto's method work better when one is aiming to pull data from studies with small treatment effects (OR close to 1), events are rare and the experimental and control groups have a similar number of participants (PANESAR, SIOW and ATHANASIOU, 2010). In other scenarios different than this, it has shown to give biased results.

The advantages and disadvantages of those methods have been pointed out (PANESAR, SIOW and ATHANASIOU, 2010). Peto's method can produce biased results when there is a mismatch in the number of participants between the experimental and control groups. If the number of studies to be included is small but their simple sizes are large, then the inverse variance-based method is the most appropriate to be employed. If an opposite situation occurs, then the Mantel-Haenszel method should be chosen. It is recommended that a continuity correction should be used (add 0.5 to each cell of a 2 x 2 table) for sparse data, except when very little heterogeneity exists among studies.

I.5.5.1.2. Random effects meta-analysis

The previous described model of the fixed effects assumes that the true effect size is the same in all studies (BORENSTEIN *et al.*, 2009). However, this assumption is not plausible for most of the systematic reviews and meta-analysis. One may assume that a group of studies have enough in common to be included in a meta-analysis, but there is generally no reason to assume that the true effect size is the same in all the studies (BORENSTEIN *et al.*, 2009). Despite studies may have addressed the same clinical question, they may differ in the demographic characteristics of the included participants and in the implementation of interventions (BORENSTEIN *et al.*, 2009). In order to address these variations across the studies, a meta-analysis should be conducted under the random effects model (BORENSTEIN *et al.*, 2009). The random effects meta-analysis assumes that each study estimates its own treatment effect, which follows a normal distribution (PANESAR, SIOW and ATHANASIOU, 2010).

The DerSimonian and Laird random effects method incorporates the assumption that the different studies are estimating different but yet related treatment effects (PANESAR, SIOW and ATHANASIOU, 2010). This method is based on the inverse variance approach used in the fixed effects model. In the fixed effects model each study is weighted by the inverse of its variance (BORENSTEIN *et al.*, 2009). In the random effects analysis too. However, the difference is that the variance now included the original (within-studies) variance plus the estimate of the between studies variance, T^2 (BORENSTEIN *et al.*, 2009). Under the random-effects model, the weight assigned to each study is

$$W_i^* = \frac{\sum_{i=1}^{k} Y_i W_i^*}{\sum_{i=1}^{k} W_i^*}$$

Where W_i^* is

$$W_i^* = \frac{1}{V_{Yi}^*}$$

When there is no heterogeneity among the studies (between studies variance = 0), the DerSimonian and Laird method and the inverse variance-based method on a fixed effects analysis will produce identical results (and thus also give results similar to the Mantel-Haenszel method) (PANESAR, SIOW and ATHANASIOU, 2010). When heterogeneity is present

(between studies variance ≠ 0), the confidence intervals of the treatment effect estimate under the DerSimonian and Laird method will be wider when compared with an estimation under a fixed effects methods and, therefore, the claims of a statistical significance will be more conservative (PANESAR, SIOW and ATHANASIOU, 2010).

It is common the researchers to conduct sensitivity analyses in order to evaluate the robustness of the meta-analysis' results. It is recommended that both fixed and random effects models should be used in such sensitivity analyses (PANESAR, SIOW and ATHANASIOU, 2010). This gives a picture of the amount of between-studies variance (heterogeneity) influencing the results and helps investigators taking conclusions.

1.5.6. CUMULATIVE META-ANALYSIS

Cumulative meta-analysis is performed by adding studies one by one, until all relevant studies have been included in the analysis (BORENSTEIN *et al.*, 2009). Cumulative meta-analysis is not a different analytical method. It only displays the results the results into a sequence based on some factor and how the estimate of the effect size shifts as a function of such factor (BORENSTEIN *et al.*, 2009). Cumulative meta-analysis is commonly used to displays the effect size estimate over time.

If conducted prospectively, cumulative meta-analyses can be updated every time a new study is published. If conducted based on study size, cumulative meta-analysis allow evaluating how small studies influence the overall result (PANESAR, SIOW and ATHANASIOU, 2010). One should note that if the objective is to assess the relationship between a factor and the estimated effect size, then the most appropriate analysis is the meta-regression.

Figure I.6 displays a forest plot where it is shown the estimated RR and its correspondent 95% confidence interval (CI) of a cumulative meta-analysis of RCTs comparing rofecoxib with control (JÜNI *et al.*, 2004). By 2000, an increased RR for myocardial infarction was already identified (RR 2,30; 1,22 - 4,33, p=0,01). Rofecoxib was withdrawn from market in 2004 due to cardiovascular adverse effects.

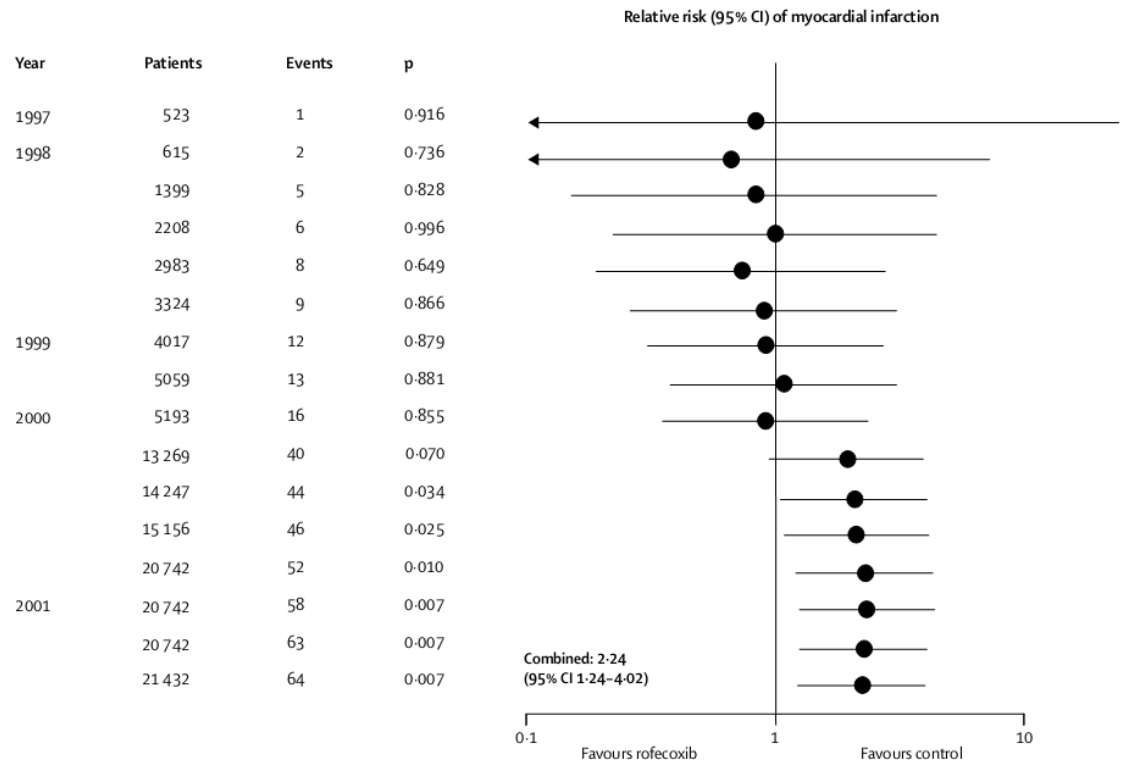


Figure I.6 - Cumulative meta-analysis of randomised trials comparing rofecoxib with control (JÜNI *et al.*, 2004).

I.5.7. META-REGRESSION

Regression can be used to assess a relationship between one or more covariates and a dependent variable (BORENSTEIN *et al.*, 2009). This approach is essentially the same that is used in meta-analysis, except that the covariates are at the level of the study rather than the level of the subject, and the dependent variable is the effect size (e.g.: RR, OR or RD) in the studies rather than subject scores (BORENSTEIN *et al.*, 2009). Using this procedure in meta-analysis is called meta-regression.

Meta-regressions usually differ from simple regressions in two ways (HIGGINS and GREEN, 2011). First, studies with larger sample size have more influence on the relationship than small sample sized studies, since studies are weighted by the precision of their respective effect estimate (HIGGINS and GREEN, 2011). Second, there is a need to assign a weight to each study and to evaluate the existence of between-studies variance (heterogeneity) in order to select the most appropriate model (fixed *versus* random effects analysis) (BORENSTEIN *et al.*, 2009).

Meta-regression will return a coefficient which is interpreted in relation to an increase or a decrease in the effect size. Borenstein and colleagues give a simple example which will help to understand, using a meta-analysis and a meta-regression conducted by Colditz and colleagues (1994) and Berkey and colleagues (1995), respectively (BORENSTEIN *et al.*, 2009). It is found a coefficient of -0,0292 for a meta-regression evaluating the association between a vaccine to prevent tuberculosis and latitude. The result means that everyone degree of latitude corresponds to a decrease of 0,0292 units in effect size.

Meta-regression has been used to evaluate the existence of heterogeneity between studies and how certain covariates could be responsible for excessive between-studies variance (BAKER *et al.*, 2009). The meta-analysis used as an example by Borenstein and colleagues estimated a RR of 0,650, 95% CI 0,601 to 0,704. The I^2 test for heterogeneity yielded a result of 92,12%, which is excessive. The studies included in the meta-analysis of Colditz and colleagues (1994) estimated RR ranging from 0,198 to 1,562. The meta-regression allowed explaining how the efficacy of the vaccine depended on the latitude.

I.5.8. HETEROGENEITY

Meta-analysis combines data across studies in order to achieve a summary effect size. The value of a meta-analysis increases when the results of the included studies show important effects of similar magnitude (HIGGINS *et al.*, 2003). However, there are several issues in the process of integrating evidence from studies, in particular when they present conflicting results (HIGGINS *et al.*, 2003; IOANNIDIS, 2008). In order to attempt assessing the consistency between the included studies, reports of meta-analyses commonly present a statistical test for heterogeneity (HIGGINS *et al.*, 2003). The test for heterogeneity aims to determine whether the differences underlying the results of the studies are genuine (heterogeneity), or whether the differences in the findings are related with chance (homogeneity) (HIGGINS *et al.*, 2003). The presence of heterogeneity can also be investigated using plots.

Heterogeneity may arise from differences in study designs, demographic characteristics of the included participants, administration mode, dosage or frequency of the interventions, duration of treatments, or methodological aspects specific to each study, as the outcomes assess procedure (IOANNIDIS, 2008; BORENSTEIN *et al.*, 2009; EUROPEAN MEDICINES AGENCY, 2001; HIGGINS and GREEN, 2011).

I.5.8.1. Statistical tests to investigate heterogeneity

Commonly used in meta-analysis, the Cochrane' Q statistic test explores the existence of heterogeneity which may be considered significant or not based on a P-value (IOANNIDIS, 2008). Formally, a null hypothesis is formulated stating that all studies share a common effect size and then test this hypothesis (BORENSTEIN *et al.*, 2009). Under the null hypothesis, the Q test follows a χ^2 distribution with degrees of freedom to *k*-1, setting a P-value for any observed value of Q (BORENSTEIN *et al.*, 2009). It is usual to use a cut-off of 10% for significance, instead of 5%, due to the poor capacity of the Q test to detect true heterogeneity (HIGGINS *et al.*, 2003). However a non-significant result may not be indicative of homogeneity (HIGGINS *et al.*, 2003; BORENSTEIN *et al.*, 2009).

The I^2 statistic test offers a quantitative measure of the heterogeneity, by estimating the percentage of total variation across studies that is due to heterogeneity rather than chance (HIGGINS *et al.*, 2003). It is computed as

$$I^2 = \frac{Q-df}{Q} \times 100$$

where Q is the χ^2 statistic and *df* is its degrees of freedom. The value of the I^2 statistic test ranges from 0 to 100%.

There is no formal categorization to classify the presence of heterogeneity as excessive or not (HIGGINS *et al.*, 2003). Many authors consider statistical heterogeneity as low when $0\% < I^2 < 25\%$, moderate when $25\% < I^2 < 50\%$ or high when $I^2 > 50\%$, although other considerations are accepted (HIGGINS and GREEN, 2011).

The I^2 statistic test gives us an estimation of the excess of between-studies variance which may influence the meta-analysis' results. However, it should be noted that this test is a descriptive statistic and not an estimate of any underlying quantity (BORENSTEIN *et al.*, 2009).

It should be noted that the model used to conduct the meta-analysis should not be chosen based on the heterogeneity test (BORENSTEIN *et al.*, 2009). In particular conducting two-stage meta-analysis, in which the meta-analytic model (fixed or random effects) is determined by the result of a statistical test. These strategies were found to be potentially misleading (HIGGINS *et al.*, 2003). The meta-analytic model should be chosen based on the investigators' understanding of whether all studies share similar methodological designs and common effect sizes (BORENSTEIN *et al.*, 2009).

I.5.8.2. Visual investigation of heterogeneity

I.5.8.2.1. L'Abbé plot

The L'Abbé plot is applicable to meta-analyses of studies with binary outcomes. The event rate in the intervention group (*y*-axis) is plotted against the event rate in the control group (*x*-axis) (FERRER, 1998). There is a central line indicating identical risks in each group (BAX *et al.*, 2009). The L'Ábbé plot may also have a regression line indicating the meta-analytic estimated size effect, usually represented by a dotted line (BAX *et al.*, 2009). Sometimes, the dots representing each study can have sizes proportional to the study weights (BAX *et al.*, 2009). If the intervention group has better results than the control group, the dots will be displayed under the central line. In the absence of heterogeneity, the dots will form a consistent band on the plot indicating a similar relationship from study to study (Figure I.7a). However, in the presence of heterogeneity, the dots may be substantially scattered (Figure I.7b).

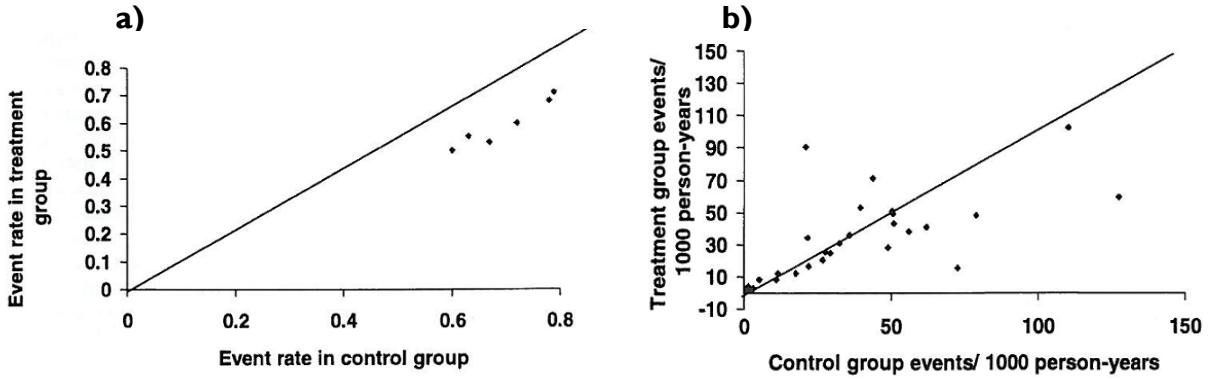


Figure I.7 - L'Abbé plots displaying the results of two meta-analyses, one suggesting lower evidence of between-studies heterogeneity (a) and other suggesting the existence of heterogeneity between the results of the studies (b) (FERRER, 1998).

I.5.8.2.2. Galbraith plot

In the Galbraith plot, the *y*-axis will show the effect size divided by its standard error and the *x*-axis will show the inverse of the standard error (BAX *et al.*, 2009). Like the L'Abbé plot, each study is represented by a single dot. A regression line representing the slope of the meta-analytic estimated size effect based on the fixed-effects model runs centrally (BAX

et al., 2009). Parallel to the regression line, two lines limit a 95% confidence interval in which most dots would be expected to fall (BAX *et al.*, 2009). These two lines are separated by a 2-standard deviation distance from the regression line. The distance between the dots and the regression line represents the extent in which each study contributes to the heterogeneity. Studies located outside the limits will be trials where the 95% confidence interval does not contain the pooled estimate and, therefore, may contribute to the excess of heterogeneity (BAX *et al.*, 2009). In the absence of heterogeneity it is expected that all studies will fall inside the 95% confidence interval limit. Figure I.8 represents a Galbraith plot where several points are located outside the limits, representing studies that may contribute to excessive heterogeneity.

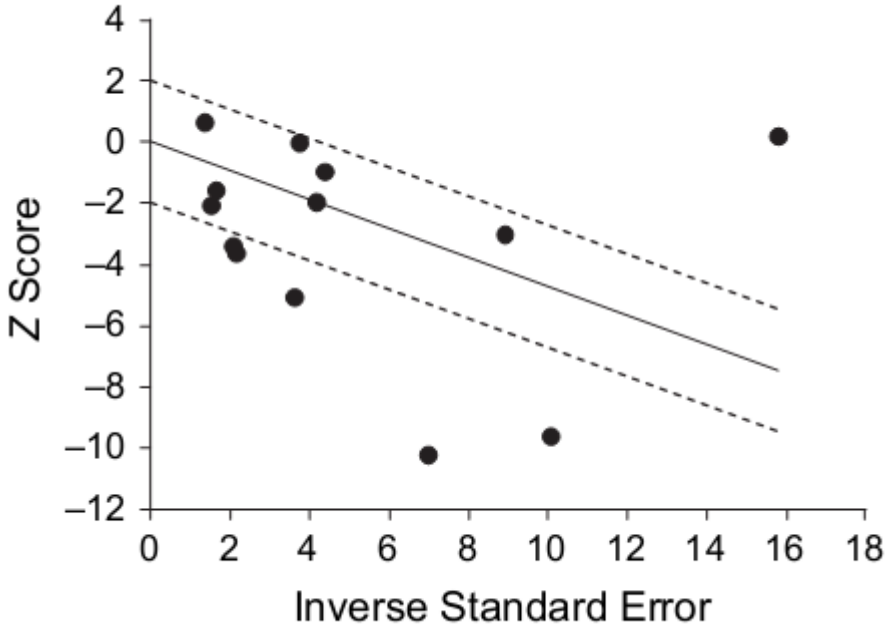


Figure I.8 - Galbraith plot (BAX et al., 2009).

I.5.9. PUBLICATION BIAS

Publication bias occurs when the studies selected to be included in a meta-analysis or systematic review do not represent all studies on the topic of interest (MACASKILL, WALTER and IRWIG, 2001). If the studies are a biased sample of the existent evidence, then the estimated effect sized will reflect this bias (BORENSTEIN *et al.*, 2009). Theoretically, the literature search conducted would be able to identify all the relevant studies addressing the

same research issue. However, this may not be possible and even with existing electronic search tools some studies fitting the inclusion criteria may escape (BORENSTEIN *et al.*, 2009). Moreover, studies with statistically significant positive results are more likely to be highlighted and easily published (MACASKILL, WALTER and IRWIG, 2001). This may induce bias in publish literature and subsequently induce bias in systematic reviews and meta-analysis (BORENSTEIN *et al.*, 2009). Other sources of bias that can affect effect sizes estimates in meta-analysis are the inclusion of studies written in specific languages or the search of a particular type databases or journals (BORENSTEIN *et al.*, 2009). Herein is described some tools which help researchers to evaluate the presence of publication bias in meta-analysis.

I.5.9.1. Funnel plot

The visual inspection of a funnel plot is one of the most common methods used to detect publication bias. The funnel plot is plotted with effect size on the *X* axis and the sample size or variance (or standard error) on the *Y* axis (BORENSTEIN *et al.*, 2009; MACASKILL, WALTER and IRWIG, 2001). The plot is expected to take a shape of a funnel in the absence of publication bias, with the amount of scatter about the true effect (a vertical line of symmetry) decreasing with increasing sample size or, if this is the case, with decreasing of standard error (MACASKILL, WALTER and IRWIG, 2001). Large studies appear located toward the top of the graph and generally near the line representing the mean effect size (BORENSTEIN *et al.*, 2009). Smaller studies tend to appear in the bottom of the graph and are usually spread across a broad range of values (BORENSTEIN *et al.*, 2009). Figure I.9 represents two funnel plots of different meta-analyses, one where publication bias was not detected (Figure I.9a) and other funnel plot suggesting the presence of publication bias (Figure I.9b) (MACASKILL, WALTER and IRWIG, 2001).

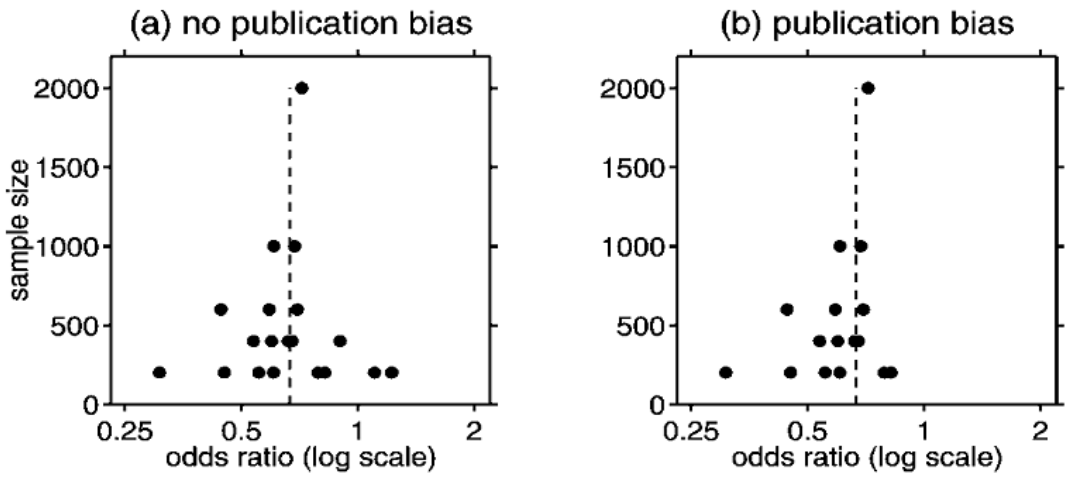


Figure I.9 - Funnel plots based on simulation of meta-analyses, one where publication bias was not detected (a) and other where publication bias was present (b) (MACASKILL, WALTER AND IRWIG, 2001).

Since the interpretation of funnel plots is subjective, other tests have been suggested to test the presence of publication bias (BORENSTEIN *et al.*, 2009).

I.5.9.2. Begg and Mazumdar rank correlation test

The Begg and Mazumdar rank correlation test assesses the significance of the correlation between the effect size estimates and the meta-analysis weights (DEEKS, MACASKILL and IRWIG, 2005). The test involves standardizing the effect size estimates to stabilize the variances (dividing each estimate by the standard errors) and performing an adjusted rank correlation test based on Kendall's tau (DEEKS, MACASKILL and IRWIG, 2005). However, this test has shown to have low power when the meta-analysis includes few studies.

I.5.9.3. Egger regression test

One of the most used tests to evaluate the presence of publication bias is the Egger regression test. The Egger regression test assesses the funnel plot asymmetry based on a regression of the standardized effect size estimates and a precision estimate (standard error), testing whether the intercept deviates from zero (DEEKS, MACASKILL and IRWIG, 2005). As an example, when outcomes are dichotomous, and intervention effects are

expressed as ORs, this approach corresponds to a linear regression of the log OR on its standard error, weighted by the inverse of the variance of the log OR (HIGGINS and GREEN, 2011). When no publication bias is present, the intercept will pass through the origin (expected value of zero) and the slope will be an unbiased estimate of the true (underlying) effect (MACASKILL, WALTER and IRWIG, 2001). On the other hand, if publication bias is present, the fitted line will not pass through the origin (MACASKILL, WALTER and IRWIG, 2001). The size of the intercept is taken as the basis of a test for publication bias. Figure I.10 presents the results of the Egger regression test, in the presence (Figure I.10a) and in the absence (Figure I.10b) of publication bias, respectively.

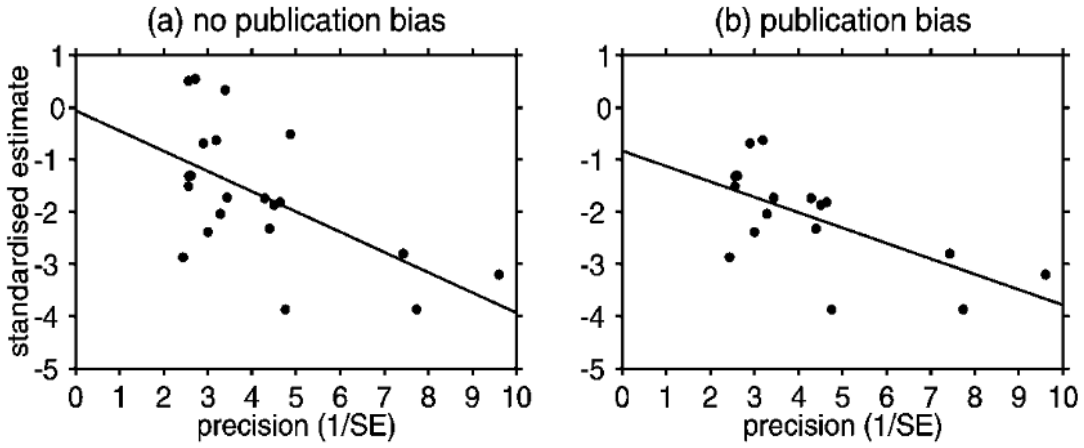


Figure I.10 - Plots showing Egger's regression test results, one where publication bias was not detected (a) and other where publication bias was present (b) (MACASKILL, WALTER and IRWIG, 2001).

I.6. THE POSTMARKETING DRUG RISK ASSESSMENT

I.6.1. THE BENEFIT-RISK ASSESSMENT PROCESS

The benefit-risk ratio assessment can be defined as the consideration of whether a drug, when prescribed or indicated to treat a specific condition, is worth the risk to the patient as compared with possible benefits. The benefit-risk ratio is evaluated in the context of two dimensions (CURTIN and SCHULZ, 2011). The dimension of benefits is measure in terms of clinical efficacy, which is the successful treatment of prevention of the condition for which the drug is being indicated or the improvement of the patients' quality of life. The observed ADRs and potential risk for unanticipated ADRs consist in the dimension of the risk. To receive market authorisation, a drug is evaluated on the basis of scientific criteria of quality, safety and efficacy for its intended use (US FOOD AND DRUG ADMINISTRATION, 2013). The assessment of the benefit-risk relation should be based on the available RCTs designed to determine the efficacy and safety of the drug when used in the intended therapeutic indication (EUROPEAN MEDICINES AGENCY, 2007). Since all drugs have the ability to cause adverse effects, a drug is considered "safe" if its benefits outweigh its risks (US FOOD AND DRUG ADMINISTRATION, 2013).

The assessment of a drug's benefit-risk ratio by a regulatory authority is essentially a qualitative procedure and relies heavily on expert panels committees' opinions (CURTIN and SCHULZ, 2011). However, the information about the drug can be somehow limited and may not address all relevant questions, introducing uncertainties in the decision-making process (US FOOD AND DRUG ADMINISTRATION, 2013). Two experts may agree on the evidence supporting the efficacy of a certain drug, they may have different opinions regarding the safety profile; one expert may say it is worthwhile to have the drug in market while other may just recognize its value where other drugs have failed. The subjectivity of the benefit-risk ratio assessment process takes into account the severity of the condition being treated, the population being treated, the effectiveness of the available treatments, the nature and severity of a specific AE as well as other factors (US FOOD AND DRUG ADMINISTRATION, 2013; MADRE *et al.*, 2006; DAL PAN and ARLETT, 2012). No method proves fully satisfactory solution regarding the benefit-risk ratio assessment, since it is difficult to reduce a multidimensional procedure to straightforward decisions (CURTIN and SCHULZ, 2011). The FDA and EMA do not use quantitative tools to conduct benefit-risk ratio assessments. Both agencies produced guidance for assessors in order to promote the transparency of the

decision process (US FOOD AND DRUG ADMINISTRATION, 2013; EUROPEAN MEDICINES AGENCY, 2007). However, the need to further research in methodologies for quantitatively assess medical interventions benefit-risk ratio has been recognized (EUROPEAN MEDICINES AGENCY, 2007).

The benefit-risk ratio assessment is a continuing procedure, starting during the drug's preclinical development and continuing during the marketing period. Results from animal models determine if a drug is candidate to be administered in humans to continue further clinical evaluation (CURTIN and SCHULZ, 2011). The dynamic nature of this process is due to new findings that can arise in every phase of drug life cycle. Unknown adverse reactions can lead drugs withdrawal from market. FDA withdrawn natalizumab in 2005 after being associated with progressive multifocal leukoencephalopathy (CURTIN and SCHULZ, 2011). However, is was remarketed in 2006 since it was recognized that natalizumab improved multiple sclerosis patients' quality of life and was more effective than the therapeutic alternatives approved at that time (US FOOD AND DRUG ADMINISTRATION, 2013). Thalidomide was also relauched to treat multiple myeloma and erythema nodosum leprosum (CURTIN and SCHULZ, 2011).

The removal of a drug from market after being associated with a rare and serious ADR has a major impact in society, since patients have become exposed to such risk. Therefore, on the interest of the public health, regulatory science has a critical role in the management of drug risk assessment during the postmarketing phase of a drug.

I.6.2. THE ASSESSMENT OF RISK DURING THE DRUGS' POSTMARKETING PHASE

During the clinical development phase, no more than hundreds or few thousands of patients are exposed to a drug. After receiving a market authorisation, a larger and more varied population will receive the medication (STAFFA and DAL PAN, 2012). The knowledge about the benefit-risk ratio of a drug increases with the everyday practice, since unknown rare and/or long-latency ADRs may occur after prolonged use, as well as those occurring in patients with comorbidities and interactions with other co-prescribed medicines (MADRE *et al.*, 2006). This additional knowledge of drugs' safety profile is subject of carefully evaluation by regulatory authorities.

The postmarket drug risk assessment can be segmented in three stages: the evidence generation, the evidence interpretation and integration, and the decision-making process (Figure I.11) (HAMMAD *et al.*, 2013). Safety signals can be generated from more than one

evidence source, like spontaneously reported ADRs, RCTs, observational studies or meta-analyses (LESTER *et al.*, 2013). Each source of evidence is then assessed as its methodological quality. Regulatory authorities may require further studies to be conducted in order to clarify the potential risks of harm hypothesized by the safety signal.

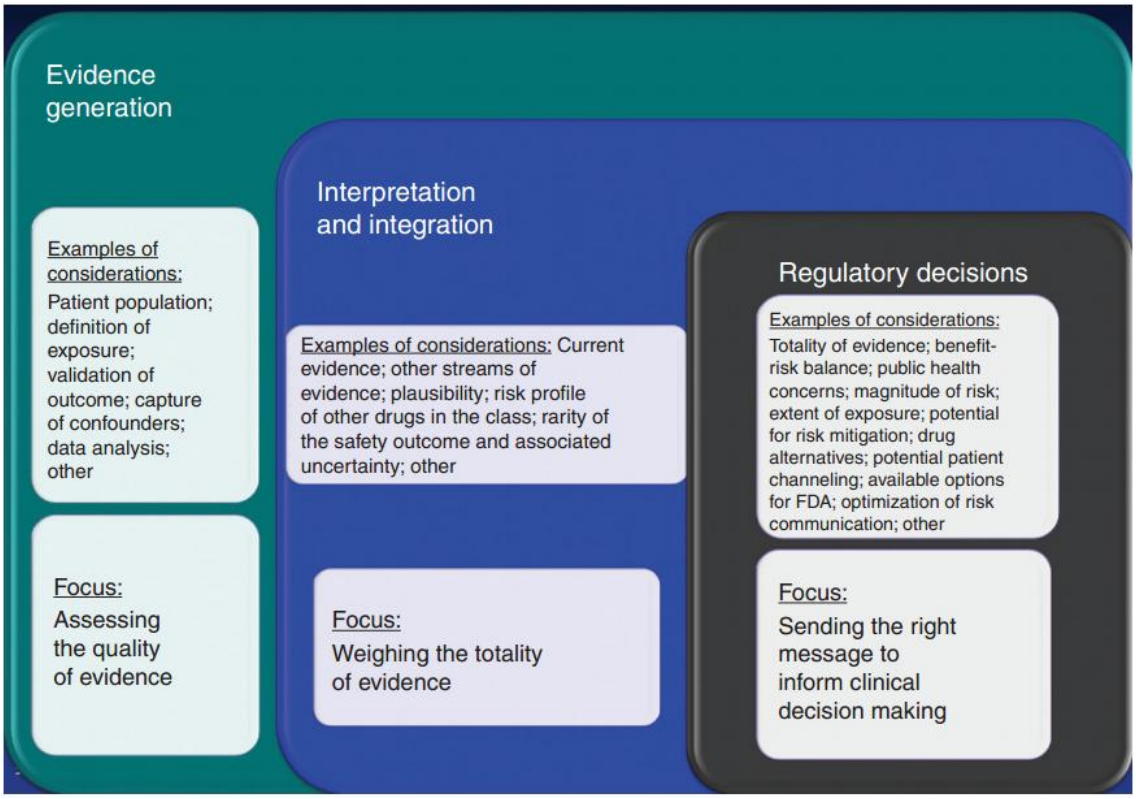


Figure I.11 - Dimensions of postmarket drug safety assessment (HAMMAD et al., 2013).

The second dimension involves weighting the contribution of each data source which supports the safety signal and to assess the level of residual uncertainty concerning the risks at the time of evaluation. For this, the causal association between the AE and the suspected drug should be assessed. The Bradford Hill's criteria, decisional algorithms and guidelines to rate evidence may be consulted by regulatory authorities in order to better interpret the available evidence (HILL, 1965; GUYATT *et al.,* 2008). Hierarchize the evidence based on the study design may be helpful (MADRE *et al.*, 2006). Descriptive studies, such case reports, case series and uncontrolled cohorts, limit the inferences that are made about causality since they are mainly used as hypothesis generating. By including a comparator group, analytic studies like RCTs, cohort or case-control, allow confirming research hypotheses and are placed in higher levels of evidence hierarchy scales (MADRE *et al.*, 2006; STROM, 2012). The amount and diversity of data sources available depends on the nature of the harm being evaluated, as well

as on the specific demographic characteristics of the population receiving treatment. Serious and rare AEs with acute onset are more likely to be spontaneously reported, an example being rhabdomyolysis associated with cerivastatin (CHANG *et al.,* 2004). In fact, since rhabdomyolysis is a rare event, spontaneously reported cases were the only data evidence on this drug-adverse effect pair (STAFFA, CHANG and GREEN, 2002). The risk for long-latency AE is better reflected in longitudinal, observational epidemiologic studies, as it was for bone fractures and proton pump inhibitors (YANG *et al.*, 2006; TARGOWNIK *et al.*, 2008).

The decision taken by the regulatory authority addressing the safety issue is considered the third dimension. The regulatory action is taken considering the entire benefit-risk relation of the drug and not only the new knowledge about the safety profile. The therapeutic position of the drug in relation to other therapeutic alternatives, the severity of the condition for which the drug is being indicated and the potential impact of the risk minimization strategies are taken into account in this stage (HAMMAD *et al.*, 2013).

The need for improvement in the regulatory science has been subject of debate. The Innovative Medicines Initiative has several projects ongoing aim to enhance the safety monitoring of drug to better support benefit-risk ratio evaluations (GOLDMAN, 2012). Since the evidence on safety issues can be generated from a diversity of sources, methods to integrate multiple studies continue to be developed (HAMMAD *et al.*, 2013).

1.6.3. COMBINE EVIDENCE ON HARMS FROM DIFFERENT DATA SOURCES

The decision-making process requires the best evidence on benefits and harms about an intervention. The methodological quality of clinical development programmes have improved over the last years (VANDENBROUCKE, 2004; SCHULZ *et al.,* 2010). At the time to grant a license to a drug, regulatory authorities are in possession of a considerable volume of knowledge about its benefit-risk profile. Nonetheless, most evidence on harms from a medical intervention is obtained from observational research since it reaches the market (VANDENBROUCKE, 2006).

Clinical trials are considered to provide the strongest evidence of efficacy regarding an intervention (LESKO and MITCHELL, 2012; PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006). The random assignment allows having comparable groups of patients and the applying statistics to explore data on the basis of random sampling (VANDENBROUCKE, 2004). This provides better control against bias than other study designs. The sample size of a RCT is powered to test differences between groups regarding an efficacy outcome. However, most

of RCTs are too small and don't have enough follow-up time to detect AEs that fewer than about one per 200/year or taking longer than one or two year to develop (VANDENBROUCKE, 2004). Yet, RCTs provide accurate information on the most common and acute AEs (LESKO and MITCHELL, 2012; VANDENBROUCKE and PSATY, 2008).

To evaluate rare and long-term AEs, longitudinal comparative observational studies such case-controls and population based cohorts are needed (STROM 2006; PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006; VANDENBROUCKE, 2006). Large-scale observational studies may be devoted to study specific AEs, unlike RCTs which may report fragmented pieces of evidence on harms which may deserve further investigation (PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006). The strict inclusion/exclusion criteria of RCTs usually exclude subgroups of patients, such as pregnant women, children, or elderly patients with or without comorbidities (MADRE *et al.*, 2006). Once in the market, observational studies are the most appropriate ones to study the medicines' safety profile since they may reflect better the nature and the frequency of AEs experienced by patients in clinical practice.

The decision-making process, whenever a physician intends to prescribe a drug in his clinical daily practice or when a regulatory authority decides to carry out a benefit-risk ratio assessment process, should combine information on the best evidence of benefits and harms (VANDENBROUCKE and PSATY, 2008). Clinical trials may constitute the most authoritative source of evidence for benefits. To evaluate safety issues, however, it is suggested that both experimental and observational evidence should be considered (VANDENBROUCKE and PSATY, 2008; AGENCY FOR HEALTHCARE RESEARCH AND QUALITY, 2014). Agency for Healthcare Research and Quality guidance on Effectiveness and Comparative Effectiveness Reviews recommends investigators to include both RCTs and observational studies aiming to assess harms when comparing medical interventions (AGENCY FOR HEALTHCARE RESEARCH AND QUALITY, 2014). Cochrane Handbook for Systematic Reviews of Interventions refers to the inclusion of nonrandomized and randomized trials when assessing adverse effects. It is strongly recommended investigators should make any attempt to combine evidence from experimental and observational data. A study conducted to assess how information about AEs is included in systematic reviews found that most of Cochrane reviews rely only on data from RCTs (HOPEWELL, WOLFENDEN and CLARKE, 2008).

Incorporate data on AEs from different sources in a systematic review process is challenging and requires proper assessment of their internal validity and possible sources of heterogeneity, as well as the necessary methods of analysis (CHOU and HELFAND, 2005). Such methodological issues are extended to meta-analysis when this methodology is

considered to integrate data from both experimental and observational studies. Criteria on how meta-analysis technique could be used to combine available information from both RCTs and observational studies in order to evaluate safety issues has been debated (COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES, 2013).

I.7. OBJECTIVES OF THIS THESIS

The meta-analytic technique can be useful as a Pharmacovigilance method to systematically evaluate emerging data on pharmacological interventions' safety profile during their development, licensing and subsequent launch onto the healthcare market. Therefore, the primary objective of this project is to identify the role of meta-analysis as a Pharmacovigilance approach and to evaluate how best to combine safety information from both experimental and observational studies through this statistical technique.

The specific objectives outlined for this project were the following:

1) To perform a systematic review aiming at identifying meta-analyses from both experimental and observational studies where safety was found to be a primary outcome measure.

2) To conduct a meta-analyses aiming at evaluating the risk of a frequent adverse event by pooling data from RCTs, and to explore the between-studies heterogeneity.

3) To conduct a meta-analyses aiming at evaluating the risk of a rare adverse event by pooling data from both experimental and observational studies, and to explore the between studies heterogeneity.

4) To identify the data sources supporting benefit/risk ratio reevaluations conducted by regulatory authorities on safety issues.

5) To evaluate how risk estimates generated from cumulative meta-analysis performs over time for drugs having their benefit/risk ratio reevaluated due to safety issues and, additionally, compare the risk estimates with regulatory authorities' conclusions.

To fullfil point 1), the study entitled "Data sources on drug safety evaluation: a review of recent published meta-analyses" was conducted (ALVES, BATEL MARQUES AND MACEDO, 2012a); to fullfil point 2), the study entitled "Apixaban and Rivaroxaban Safety After Hip and Knee Arthroplasty: A Meta-Analysis" was conducted (ALVES, BATEL MARQUES AND MACEDO, 2012b); to fullfil point 3), the study entitled "A meta-analysis of serious adverse events

reported with exenatide and liraglutide: Acute pancreatitis and cancer" was conducted (ALVES, BATEL MARQUES AND MACEDO, 2012c); to fullfil point 4), the study entitled "Sources of information used by regulatory agencies on the generation of drug safety alerts" was conducted (ALVES, MACEDO AND BATEL MARQUES, 2013); to fullfil point 5), the study entitled "Drug-safety alerts issued by regulatory authorities: usefulness of meta-analysis in predicting risks earlier" was conducted (ALVES, BATEL MARQUES AND MACEDO, 2014).

I.8. BIBLIOGRAPHY

AGENCY FOR HEALTHCARE RESEARCH AND QUALITY. – Methods Guide for Effectiveness and Comparative Effectiveness Reviews. AHRQ Publication No. 10(14)-EHC063-EF. Rockville, MD, USA, January 2014.

AHMAD, S.R.; MARKS, N.S.; GOETSCH, R.A. – **Spontaneous reporting in the United States.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 91-116.

AKICI, A.; OKTAY, S. – Rational Pharmacotherapy and Pharmacovigilance. **Curr Drug Saf.** 2;1 (2007) 65-69.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Data sources on drug safety evaluation: a review of recent published meta-analyses. **Pharmacoepidemiol Drug Saf.** 21:1 (2012a) 21-33.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Apixaban and Rivaroxaban Safety After Hip and Knee Arthroplasty: A Meta-Analysis. **J Cardiovasc Pharmacol Ther.** 17:3 (2012b) 266-276.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. **Diabetes Res Clin Pract.** 98:2 (2012c) 271-284.

ALVES, C.; MACEDO, A.F.; MARQUES, F.B. – Sources of information used by regulatory agencies on the generation of drug safety alerts. **Eur J Clin Pharmacol.** 69:12 (2013) 2083-2094.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Drug-safety alerts issued by regulatory authorities: usefulness of meta-analysis in predicting earlier risks. **Eur J Clin Pharmacol.** 2014 DOI:10.1007/s00228-014-1670-5.

AMGEN – Safety and Efficacy Study to Evaluate Denosumab Compared With Zoledronic Acid in Postmenopausal Women With Osteoporosis. (2012) Available from: http:// http://clinicaltrials.gov/ct2/show/study/NCT01732770. NLM Identifier: NCT01732770

ANONYMOUS. – Aspirin after myocardial infarction. **Lancet.** I (1980) 1172-1173.

BAHRI, P.; TSINTIS, P.; WALLER, P.C. – **Regulatory Pharmacovigilance in the EU.** In: MANN, Ronald; ANDREWS, Elizabeth., Pharmacovigilance. 2nd Ed., Chichester, England: John Wiley & Sons, 2007. ISBN: 978-0-470-01803-3. p. 185-198.

BAKER, W.L. [et al] – Understanding heterogeneity in meta-analysis: the role of meta-regression. **Int J Clin Pract.** 63;10 (2009) 1426-1434.

BAX, L. [et al] – More than numbers: the power of graphs in meta-analysis. **Am J Epidemiol.** 169;2 (2009) 249-255.

BEIJER, H.J.M.; DE BLAEY, C.J. – Hospitalisations caused by adverse drug reactions (ADR): a meta-analysis of observational studies. **Pharm World Sci.** 24;2 (2002) 46-54.

BERKEY, C.S. [et al] – A random-effects regression model for meta-analysis. **Stat Med.** 14;4 (1995) 395-411.

BERLIN, J.; SOLEDAD CEPEDA, M.; KIM, C. –**The Use of Meta-analysis in Pharmacoepidemiology** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 723-756.

BOMBARDIER, C. [et al] – Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. **N Engl J Med.** 343;21 (2000) 1520-1528.

BORENSTEIN, M. [et al] – **Introduction to meta-analysis.** Chichester, England: John Wiley & Sons, 2009. ISBN 978-0-470-05724-7.

CABRITA DA SILVA, J.; SOARES, M.A.; MARTINS, S.O. – Reações Adversas a Medicamentos: Análise da base de dados do Sistema Nacional de Farmacovigilância (SVIG) 2009-2011. Relatório Final. (2012) Available from https://www.infarmed.pt/portal/page/portal/INFARMED/MEDICAMENTOS_USO_HUMANO/FARMACOVIGILANCIA/Relatorio_analise_dados_SVIG_2009_2011.pdf.

CHALMERS, I.; ALTMAN, D.I. – Systematic Review. London, England: BMJ Publishing Group, 1995, ISBN 0-7279-0904-5.

CHALMERS, I. [et al] – A method for assessing the quality of a randomized control trial. **Control Clin Trials.** 2;1 (1981) 31-49.

CHANG, J.T. [et al] – Rhabdomyolysis with HMG-CoA reductase inhibitors and gemfibrozil combination therapy. **Pharmacoepidemiol Drug Saf.** 13;7 (2004) 417-426.

CHOU, R.; HELFAND, M. – Challenges in systematic reviews that assess treatment harms. **Ann Intern Med**.142;12 Pt 2 (2005) 1090-1099.

COLDITZ, G.A. [et al] – Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. **JAMA**. 271;9 (1994) 698-702.

COMMISSION DIRECTIVE 2010/84/EU of 15 December 2010, as regards pharmacovigilance, Directive 2001/83/EC on the Community code relating to medicinal products for human use EC. 2010, Official Journal of the European Union. p. L 348/74 - L 348/99.

COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES – CIOMS Working Group X on "Considerations for applying good meta-analysis practices to clinical safety data within the biopharmaceutical regulatory process". (2013) Available from http://www.cioms.ch/index.php/2012-06-10-08-47-53/working-groups/working-group-x.

CURTIN, F.; SCHULZ, P. – Assessing the benefit: risk ratio of a drug--randomized and naturalistic evidence. Dialogues Clin Neurosci. 13;2 (2011) 183-90.

DAL PAN, G.; ARLETT, P. – **The Role of Pharmacoepidemiology in Regulatory Agencies.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 107-116.

DAL PAN, G.; LINDQUIST, M.; GELPERIN, K. – **Postmarketing Spontaneous Pharmacovigilance Reporting Systems.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 1371-57.

DEEKS, J.J. [et al] – Evaluating non-randomised intervention studies. **Health Technol Assess.** 7;27 iii-x (2003) 1-173.

DEEKS, J.J.; MACASKILL, P.; IRWIG, L. – The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. **J Clin Epidemiol.** 58:9 (2005) 882-893.

DETSKY, A.S. [et al] – Incorporating variations in the quality of individual randomized trials into meta-analysis. **J Clin Epidemiol.** 45;3 (1992) 255-265.

DOWNS, S.H.; BLACK, N. – The feasibility of creating a checklist for assessment of the methodological quality of both the randomised and nonrandomised studies of health care interventions. **J Epidemiol Community Health.** 52 (1998) 377-384.

EBBESEN, J. [et al] – Drug-related deaths in a department of internal medicine. **Arch Intern Med.** 161;19 (2001) 2317-2323.

EDWARDS, I.R. [et al] – **Global Drug Surveillance: The WHO Programme for International Drug Monitoring.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 117-136.

ELWOOD, P.C. [et al] – A randomised controlled trial of acetyl salicylic acid in the secondary prevention of mortality from myocardial infarction. **BMJ.** 1 (1974) 436-440.

ERNST, F.R.; GRIZZLE, A.J. – Drug-related morbidity and mortality: updating the cost-of-illness model. **J Am Pharm Assoc (Wash).** 41;2 (2001) 192-199.

ESSEBAG, V. [et al] – The nested case-control study in cardiology. **Am Heart J**. 146;4 (2003) 581-590.

EUROPEAN MEDICINES AGENCY – Guideline on good pharmacovigilance practices (GVP) – Module VIII (Rev 1). EMA/813938/2011 Rev 1, 2013.

EUROPEAN MEDICINES AGENCY – Mandate, objectives and rules of procedure for the CHMP Pharmacovigilance Working Party (adopted by the PhVWP on 21 June 2005 and the CHMP on 23 June 2005). EMEA/CHMP/PhVWP/88786/04, 2005.

EUROPEAN MEDICINES AGENCY – New EU pharmacovigilance legislation - Key concepts. EMA/186974/2012, 2013.

EUROPEAN MEDICINES AGENCY – Note for guidance on definitions and standards for expedited reporting. CPMP/ICH/3945/03, Committee for Proprietary Medicinal Products (CPMP), 2003.

EUROPEAN MEDICINES AGENCY – Note for guidance on planning pharmacovigilance activities. CPMP/ICH/5716/03, Committee for Proprietary Medicinal Products (CPMP), 2005.

EUROPEAN MEDICINES AGENCY – Points to consider on application with 1. Meta-analysis; 2. One pivotal study. CPMP/EWP/2330/99, Committee for Proprietary Medicinal Products (CPMP), 2001.

EUROPEAN MEDICINES AGENCY – Reflection paper on benefit-risk assessment methods in the context of the evaluation of marketing authorisation applications of medicinal products for human use. EMEA/CHMP/15404/2007, Committee for Medicinal Products for Human Use (CHMP), 2007.

EUROPEAN NETWORK OF CENTRES FOR PHARMACOEPIDEMIOLOGY AND PHARMACOVIGILANCE – About Us. (2014) Available from http://www.encepp.eu/structure/index.shtml.

FERRER, R.L. – Graphical methods for detecting bias in meta-analysis. **Fam Med.** 30; 8 (1998) 579-583.

FROST, W.H. – The outlook for the eradication of tuberculosis. **Am Rev Tuberc.** 32 (1935) 644-650.

GANDHI, T.K. [et al] – Adverse events in ambulatory care. **N Engl J Med.** 348;16 (2003) 1556-1564.

GLASS, G.V. – Primary, secondary and meta-analysis of research. **Educ Researcher.** 10 (1976) 3-8.

GLIKLICH, R.E.; DREYER, N.A. – **Registries for Evaluating Patient Outcomes: A User's Guide.** 2nd Ed. Rockville, MD, USA: Agency for Healthcare Research and Quality, 2010, AHRQ Publication No.10-EHC049.

GOLDMAN, M. – The innovative medicines initiative: a European response to the innovation challenge. **Clin Pharmacol Ther.** 91;3 (2012) 418-425.

GRIMES, D.A.; SCHULZ, K.F. – Cohort studies: marching towards outcomes. **Lancet**. 359;9303 (2002) 341-345.

GRIMSHAW, J. – So what has the Cochrane Collaboration ever done for us? A report card on the first 10 years. **CMAJ.** 171;7 (2004) 747-749.

GUYATT, G.H. [et al] – GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. **BMJ**. 336;7650 (2008) 924-926.

GYLLENSTEN, H. [et al] – Modelling drug-related morbidity in Sweden using an expert panel of pharmacists'. **Int J Clin Pharm.** 34;4 (2012) 538-546.

HALL, G.C. [et al] – Guidelines for good database selection and use in pharmacoepidemiology research. **Pharmacoepidemiol Drug Saf**. 21;1 (2012) 1-10.

HAMMAD, T.A. [et al] – The future of population-based postmarket drug risk assessment: a regulator's perspective. **Clin Pharmacol Ther.** 94;3 (2013) 349-358.

HÄRMARK, L.; VAN GROOTHEEST, A.C. – Pharmacovigilance: methods, recent developments and future perspectives. **Eur J Clin Pharmacol.** 64;8 (2008) 743-52.

HAUBEN, M.; ARONSON, J.K. – Defining 'signal' and its subtypes in pharmacovigilance based on a systematic review of previous definitions. **Drug Saf.** 32;2 (2009) 99-110.

HAZELL, L.; SHAKIR, S.A.W. – Under-reporting of adverse drug reactions a systematic review. **Drug Saf.** 29;5 (2006) 385-396.

HIGGINS, J.P. [et al] – Measuring inconsistency in meta-analyses. **BMJ**. 327;7414 (2003) 557-560.

HIGGINS, J.P.T.; GREEN, S. – Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

HILL, A.B. – The environment and disease: association or causation? **Proc R Soc Med.** 58 (1965) 295-300.

HOPEWELL, S.; WOLFENDEN, L.; CLARKE, M. – Reporting of adverse events in systematic reviews can be improved: survey results. **J Clin Epidemiol** 61;6 (2008) 597-602.

INTERNATIONAL SOCIETY FOR PHARMACOEPIDEMIOLOGY – International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE). (2014) Available from https://www.pharmacoepi.org/about/history.cfm.

INTERNATIONAL SOCIETY OF PHARMACOVIGILANCE – About ISOP. (2014) Available from http://www.isoponline.org/index.php?page=about-isop.

IOANNIDIS, J.P. – Interpretation of tests of heterogeneity and bias in meta-analysis. **J Eval Clin Pract.** 14;5 (2008) 951-957.

JADAD, A.R. [et al] – Assessing the quality of reports of randomized clinical trials: is blinding necessary? **Control Clin Trials.** 17;1 (1996) 1-12.

JÜNI, P.; ALTMAN, D.G.; EGGER, M. – Systematic reviews in health care: Assessing the quality of controlled clinical trials. **BMJ.** 323;7303 (2001) 42-46.

JÜNI, P. [et al] – Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. **Lancet**. 364;9450 (2004) 2021-2029.

JÜNI, P. [et al] – The hazards of scoring the quality of clinical trials for meta-analysis. **JAMA.** 282;11 (1999) 1054-1060.

JUNTTI-PATINEN, L.; NEUVONEN. P.J. – Drug-related deaths in a university central hospital. **Eur J Clin Pharmacol.** 58;7 (2002) 479-482.

KANJANARAT, P. [et al] – Nature of preventable adverse drug events in hospitals: a literature review. **Am J Health Syst Pharm.** 60;17(2003) 1750-1759.

KAUFMAN, D.W. [et.al] – Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. **JAMA.** 287;3(2002) 337-344.

KIM, C.J.; BERLIN, J.A. – The use of meta-analysis in pharmacoepidemiology**.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 353-366.

KJAERGARD, L.L.; VILLUMSEN, J.; GLUUD, C. – Reported methodologic quality and discrepancies between large and small randomized trials in meta-analyses. **Ann Intern Med.** 135;11 (2001) 982-989.

LAYTON, D.; SHAKIR, S. – **Prescription-Event Monitoring.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 301-330.

LAZAROU, J.; POMERANZ, B.H.; COREY, P.N. – Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. **JAMA.** 279;15(1998) 1200-1205.

LEENDERTSE, A.J. [et al] – The relationship between study characteristics and the prevalence of medication-related hospitalizations: a literature review and novel analysis. **Drug Saf.** 33;3 (2010) 233-244.

LESKO, S.; MITCHELL, A. – An assessment of the safety of pediatric ibuprofen. A practitioner-based randomized clinical trial. **JAMA.** 273;12 (1995) 929-933.

LESKO, S.; MITCHELL, A. – **The Use of Randomized Controlled Trials for Pharmacoepidemiologic Studies.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 640-654.

LESTER, J. [et al] – Evaluation of FDA safety-related drug label changes in 2010. **Pharmacoepidemiol Drug Saf**. 22;3 (2013) 302-305.

LINDQUIST, M. – The need for definitions in pharmacovigilance. **Drug Saf.** 30;10 (2007) 825-830.

MACASKILL, P.; WALTER, S.D.; IRWIG, L. – A comparison of methods to detect publication bias in meta-analysis. **Stat Med.** 20;4 (2001) 641-654.

MADRE, L.K. [et al] – **Views from Academia, Industry, and Regulatory Agencies.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 63-88.

MANTEL, N. – Synthetic retrospective studies and related topics. Biometrics. 29 (1973) 479-486.

MCBRIDE, W.G. – Thalidomide and congenital abnormalities. **Lancet.** 278;7216 (1961) 1358.

MEDICINES AND HEALTHCARE PRODUCTS REGULATORY AGENCY – History of the Yellow Card Scheme. (2013) Available from http://www.mhra.gov.uk/Safetyinformation/Howwemonitorthesafetyofproducts/Medicines/TheYellowCardScheme/Historyofthescheme/index.htm

MOHER, D. [et al] – Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? **Lancet.** 352;9128 (1998) 609-613.

MOHER, D. [et al] – Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. **Lancet.** 354;9193 (1999) 1896-1900.

MOHER, D. [et al] – Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. **BMJ**. 339 (2009) b2535. doi: 10.1136/bmj.b2535.

MOJA, L.P. [et al] – Assessment of methodological quality of primary studies by systematic reviews: results of the metaquality cross sectional study. **BMJ.** 330;7499 (2005) 1053.

MOORE, N. – The past, present and perhaps future of pharmacovigilance: homage to Folke Sjoqvist. **Eur J Clin Pharmacol.** 69; Suppl 1 (2013) S33-S41.

MOORE, T.J.; COHEN, M.R.; FURBERG, C.D. – Serious Adverse Drug Events Reported to the Food and Drug Administration, 1998-2005. **Arch Intern Med.** 167;16 (2007) 1752-1759.

O'ROURKE, K. – An historical perspective on meta-analysis: dealing quantitatively with varying study results. **J R Soc Med.** 100;12 (2007) 579-582.

PAL, S. [et al] – **The World Medicines Situation 2011. Pharmacovigilance and Safety of Medicines.** 3rd Ed.,Geneva, Switzerland: World Health Organization, 2011. ISBN:

PAL, S.N. [et al] – WHO Strategy for Collecting Safety Data in Public Health Programmes: Complementing Spontaneous Reporting Systems. **Drug Saf.** 36;2 (2013) 75-81.

PANESAR, S.S.; SIOW, W.; ATHANASIOU, T. – Systematic Reviews and Meta-analyses in Surgery. In: ATHANASIOU, Thanos; DEBAS, Haile; DARZI, Ara, Key Topics in Surgical Research and Methodology. New York, USA: Springer, 2010, ISBN: 978-3-540-71914-4. p. 375-397.

PANETH, N.; SUSSER, E.; SUSSER, M. – Origins and early development of the case-control study: Part 2, The case-control study from Lane-Claypon to 1950. **Soz Praventivmed.** 47;6 (2002) 359-365.

PAPANIKOLAOU, P.N.; CHRISTIDI, G.D.; IOANNIDIS, J.P. – Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. **CMAJ**. 174;5 (2006) 635-641.

PATSOPOULOS, N.A.; ANALATOS, A.A.; IOANNIDIS, J.P. – Relative citation impact of various study designs in the health sciences. **JAMA**. 293;19 (2005) 2362-2366.

PEARSON, K. – Report on certain enteric fever inoculation statistics. **BMJ.** 3 (1904) 1243-1246.

PRENTICE, R.L. – A case-cohort design for epidemiologic cohort studies and disease prevention trials. **Biometrika.** 73 (1986) 1-12.

RÄGO, L.; SANTOSO, B. – **Drug Regulation: History, Present and Future.** In: VAN BOXTEL, C.J.; SANTOSO, B.; EDWARDS, I.R., Drug Benefits and Risks: International Textbook of Clinical Pharmacology. Revised 2nd Ed. Amsterdam, The Netherlands: IOS Press and Uppsala Monitoring Centre, 2008, ISBN: 978-1-58603-880-9. p. 65-77.

RAWLINS, M.D. – The challenge to pharmacoepidemiology. **Pharmacoepidemiol Drug Saf** 4;1 (1995) 5-9.

ROSENBERG, L.; COOGAN, P.; PALMER, J. – **Case-Control Surveillance.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 287-300.

ROSENSTOCK, J. [et al] – Dose-ranging effects of canagliflozin, a sodium-glucose cotransporter 2 inhibitor, as add-on to metformin in subjects with type 2 diabetes. **Diabetes Care.** 35;6 (2012) 1232-1238.

SCHNEEWEISS, S.; SUISSA, S. – **Advanced Approach to Controlling Confounding in Pharmacoepidemiologic Studies.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 868-892.

SCHULZ, K.F. [et al] – CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. **Ann Intern Med.** 152;11 (2010) 726-732.

SCHULZ, K.F.; GRIMES, D.A. – Case-control studies: research in reverse. **Lancet**. 359;9304 (2002) 431-434.

SHAKIR, S.A.W. – **PEM in the UK.** In: MANN, Ronald; ANDREWS, Elizabeth., Pharmacovigilance. 2nd Ed., Chichester, England: John Wiley & Sons, 2007. ISBN: 978-0-470-01803-3. p. 307-316.

SHAKIR, S.A.W. – **Prescription-Event Monitoring**. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 151-166.

SONG, J.W.; CHUNG, K.C. – Observational studies: cohort and case-control studies. **Plast Reconstr Surg.** 126;6 (2010) 2234-2242.

STAFFA, J.A.; CHANG, J.; GREEN, L. – Cerivastatin and reports of fatal rhabdomyolysis. **N Engl J Med.** 346;7 (2002) 539-540.

STAFFA, J.A.; DAL PAN, G.J. – Regulatory innovation in postmarketing risk assessment and management. **Clin Pharmacol Ther.** 91;3 (2012) 555-7.

STANLEY, K. – Design of randomized controlled trials. **Circulation.** 115 (2007) 1164-1169.

STARK, R.G.; JOHN, J.; LEIDL, R. – Health care use and costs of adverse drug events emerging from outpatient treatment in Germany: a modelling approach. **BMC Health Serv Res.** 2011 (2011) doi: 10.1186/1472-6963-11-9.

STARR, M. [et al] – The origins, evolution, and future of The Cochrane Database of Systematic Reviews. **Int J Technol Assess.** 25;S1 (2009) 182-195.

STROM, B. – **Basic Principles of Clinical Epidemiology Relevant to Pharmacoepidemiologic Studies.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 38-51.

STROM, B. – **Overview of Automated Databases in Pharmacoepidemiology**. In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012a, ISBN: 978-0-470-65475-0. p. 158-162.

STROM, B. [et al] – The Ziprasidone Observational Study of Cardiac Outcomes (ZODIAC): design and baseline subject characteristics. **J Clin Psychiatry.** 69;1 (2008) 114-121.

STROM, B.L. – **Overview of Automated Databases in Pharmacoepidemiology**. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006b, ISBN: 978-0-470-02925-1. p. 167-172.

STROM, B.L. – **Study designs available from pharmacoepidemiology studies.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 13-24.

STROM, B.L. – **What is pharmacoepidemiology?** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006a, ISBN: 978-0-470-02925-1. p. 1-2.

STROUP, D.F. [et al] – Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. **JAMA**. 283;15 (2000) 2008-2012.

SUISSA, S. – **Novel approaches to pharmacoepidemiology study design and statistical analysis**. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 383-396.

SUTTON, A.J.; HIGGINS, J.P. – Recent developments in meta-analysis. **Stat Med.** 27;5 (2008) 625-650.

TARGOWNIK, L.E. [et al] – Use of proton pump inhibitors and risk of osteoporosis-related fractures. **CMAJ**. 179;4 (2008) 319-326.

THE COCHRANE COLLABORATION – About Us. The Cochrane Collaboration, 2013b. [Acedido a 09-02-2014]. Disponível na Internet: http://www.cochrane.org/about-us

THE COCHRANE COLLABORATION – Chronology of significant events and milestones in Cochrane's history. The Cochrane Collaboration, 2013a. [Acedido a 09-02-2014]. Disponível na Internet: http://www.cochrane.org/about-us/history

THOMSEN, L.A. [et al] – Systematic review of the incidence and characteristics of preventable adverse drug events in ambulatory care. **Ann Pharmacother.** 41;9 (2007) 1411-1426.

US FOOD AND DRUG ADMINISTRATION – Biologics Centennial: 100 Years of Biologics Regulation. (2009b) Available from

http://www.fda.gov/AboutFDA/WhatWeDo/History/ProductRegulation/SelectionsFromFDLIUpdateSeriesonFDAHistory/ucm091754.htm.

US FOOD AND DRUG ADMINISTRATION – Guidance for Industry: Postmarketing Studies and Clinical Trials – Implementation of Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act. FDAAA Title IX Working Group, 2011.

US FOOD AND DRUG ADMINISTRATION – Milestones of Drug Regulation in the United States. (2009c) Available from http://www.fda.gov/AboutFDA/WhatWeDo/History/FOrgsHistory/CDER/CenterforDrugEvaluationandResearchBrochureandChronology/ucm114463.htm.

US FOOD AND DRUG ADMINISTRATION – Science and the Regulation of Biological Products. (2009a) Available from http://www.fda.gov/AboutFDA/WhatWeDo/History/ProductRegulation/100YearsofBiologicsRegulation/ucm070022.htm#TheSt.LouisTragedyandEnactmentofthe1902BiologicsControlAct

US FOOD AND DRUG ADMINISTRATION – Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making. Draft Patient Drug User Free Act (PDUFA) I Implementation Plan. (2013) Available from http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf.

VANDENBROUCKE, J.P. – What is the best evidence for determining harms of medical treatment? **CMAJ** 174;5 (2006) 645-646.

VANDENBROUCKE, J.P. – When are observational studies as credible as randomised trials? **Lancet**. 363;9422 (2004) 1728-1731.

VANDENBROUCKE, J.P.; PSATY, B.M. – Benefits and risks of drug treatments: how to combine the best evidence on benefits with the best data about adverse effects. **JAMA**. 300;20 (2008) 2417-2419.

VENULET, J.; HELLING-BORDA, M. – WHO's international drug monitoring--the formative years, 1968-1975: preparatory, pilot and early operational phases. **Drug Saf.** 33;7 (2010) e1-e23.

VOLMINK, J. [et al] – Research synthesis and dissemination as a bridge to knowledge management: the Cochrane Collaboration. **Bull World Health Organ.** 82;10 (2004) 778-783.

Wells, G.A. [et al] – The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. (2014) Available from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

WHO COLLABORATING CENTRE FOR INTERNATIONAL DRUG MONITORING – **The Importance of Pharmacovigilance. Safety monitoring of medicinal products.** Geneva, Switzerland: World Health Organization, 2002. ISBN 92-4-159015-7.

WOOD, L. [et al] – Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. **BMJ.** 336;7644 (2008) 601-605.

WORLD HEALTH ORGANIZATION – A practical handbook on the pharmacovigilance of medicines used in the treatment of tuberculosis. Enhancing the safety of the tb patient. Geneva, Switzerland: WHO Press, 2012, ISBN: 978-92-4-150349-5.

YANG, Y.X. – Long-term proton pump inhibitor therapy and risk of hip fracture. **JAMA**. 296;24 (2006) 2947-2953.

CHAPTER II – DATA SOURCES ON DRUG SAFETY EVALUATION: A REVIEW OF RECENT PUBLISHED META-ANALYSES

II. DATA SOURCES ON DRUG SAFETY EVALUATION: A REVIEW OF RECENT PUBLISHED META-ANALYSES

II.1. ABSTRACT

Meta-analysis is a quantitative approach to summarize the findings from several studies and has been applied with increasing frequency to RCTs. Because of their sample size and duration limitations, experimental studies could not be able to detect late or rare AEs, which may be identified in well-designed observational studies. This study aims to identify and analyze meta-analyses from both experimental and observational studies where safety was found to be an outcome measure. The meta-analyses inclusion criteria was established as at least one AE as primary outcome. Safety outcomes were considered as the increase in the risk for an AE after a pharmacological intervention. A MEDLINE search for meta-analyses published in the New England Journal of Medicine, The Lancet, Journal of American Medical Association, British Medical Journal, Annals of Internal Medicine, PLoS Medicine, Annual Review of Medicine, and Archives of Internal Medicine, between October 2005 and September 2010, was carried out. Sixty meta-analyses met the inclusion criteria. Of these, 53 included only experimental studies, 4 included both experimental and observational studies, and 2 included only observational studies. Of the 6 meta-analyses that included observational studies, 4 included cohort and case–control studies, and 2 included cohort, case-control, and cross-sectional studies. One meta-analysis did not report the type of studies included. Experimental studies were found to be the main source of meta-analyses on drug safety. The role of meta-analyses in pharmacovigilance is a matter of ongoing debate, and efforts are being made to develop guidelines on the use of meta-analysis in drug safety assessments, to better combine evidence about harms.

II.2. INTRODUCTION

Medicine use is a fundamental component of health care, and the optimization of drug prescribing has become an important public health problem worldwide (BATES, 1995; AKICI and OKTA, 2007). It is now becoming clear that, to assess the overall effect of medical interventions, adverse effects should be reviewed with similar rigour as therapeutic benefits (HOPEWELL, WOLFENDEN and CLARKE, 2008; CANONICO *et al.*, 2008; HICKS *et al.*, 2008).

Meta-analysis is a systematic approach to identify, synthesize, and combine the results of selected studies (DERSIMONIAN and LAIRD, 1986; KIM and BERLIN, 2006). It is used to identify sources of variation among study findings and to provide an overall measure of effect to reach conclusions about a body of research. The meta-analytic technique has been applied with increasing frequency to RCTs, which are considered to provide the strongest evidence of efficacy regarding an intervention (STROM, 2006; VANDENBROUCKE, 2004a). This is due to the fact that randomized controlled designs have better control and protection against bias than other study designs.

However, evidence on AEs reported by RCTs can be considered insufficient at some point (VANDENBROUCKE and PSATY, 2008; IOANNIDIS *et al.*, 2004). Clinical trials are able to identify the most frequent and common AEs that occurred during the intervention administration (MADRE *et al.*, 2006). However, given the relatively small sample size, their average duration, and the homogeneity of the studied population, RCTs are less likely to detect rare or long-term AEs (IOANNIDIS and LAU, 2001; VANDENBROUCKE, 2004b).

Data from observational studies can be helpful to evaluate the safety of an intervention (LOKE, DERRY and ARONSON, 2004). In some situations, the best evidence regarding the safety of minority or underrepresented populations in RCTs, such as pregnant women, children, and elderly patients with or without comorbidities, is provided from observational studies (MADRE *et al.*, 2006; ROTHWELL, 2005). The observational designs may reflect better the nature and the frequency of AEs experienced by patients in clinical practice, especially rare or late AEs (PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006).

The investigation of uncommon or long-term AEs associated with pharmacological interventions is an important application of meta-analysis. The use of meta-analysis to integrate data from different study designs can be affected by inherent biases of the considered studies (KIM and BERLIN, 2006). This study aims to identify and analyze meta-analyses from both experimental and observational studies where safety was found to be an

outcome measure in the highest ranked general/internal medicine journals, which are more likely to influence clinicians' perceptions on medicines benefit/risk ratios.

II.3. METHODS

An electronic search was carried out in MEDLINE to identify all the meta-analyses published over a period of 5 years (October 2005 to September 2010) in the New England Journal of Medicine, The Lancet, Journal of American Medical Association (JAMA), British Medical Journal (BMJ), Annals of Internal Medicine, PLoS Medicine, Annual Review of Medicine, and Archives of Internal Medicine. These journals were selected according to their impact factor. They are the eight higher ranked journals in the category of "general and/or internal medicine", according to the Science Citation Index (THOMPSON REUTERS, 2014). Search was limited to meta-analyses. The search strategy is listed in the Supplemental Data II.1.

Two researchers independently screened by hand the titles and abstracts and selected full papers for inclusion. Discrepancies were resolved by consensus involving a third investigator. Meta-analyses were included if they meet the following inclusion criteria: (i) to assess the risk for the occurrence of at least one AE related to a pharmacological intervention as a primary outcome; and (ii) to pool the results using the meta-analytic technique. Meta-analyses combining study summary results and meta-analyses combining individual patient data were eligible for inclusion. We considered drugs or medical devices eluting drugs as pharmacological interventions. An AE is de fined as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment" (EUROPEAN MEDICINES AGENCY, 1995). For the purpose of the study, safety outcomes were considered as the increase in the risk for at least one AE after a pharmacological intervention. Therefore, we restricted the study to meta-analysis of increased risk for an AE because when an AE is lower on treatment than on control, which is still an AE but also may constitute an efficacy end-point. Meta-analyses that evaluated efficacy outcomes besides safety out-comes were included if the risk for the occurrence of any AE was a primary outcome.

The following information from each meta-analysis was extracted: (i) nature of the intervention; (ii) outcomes assessed – one specific or several; (iii) resources searched and if the search strategy was reported or not; (iv) type of meta-analysis design – safety and/or efficacy; (v) type of studies included and their publication status; (vi) assessment of publication bias; (vii) methodological quality assessment of the included studies; and (viii) use of meta-analysis reporting guidelines. Additionally, for meta-analyses that included both

experimental and observational studies, we evaluated if the pooled results were different and, if so, in which direction.

Meta-analyses that evaluated the same clinical question were analyzed. Two meta-analyses were considered to be evaluating the same clinical question when, for a common study aim, they assessed the same safety out-come for the same pharmacological intervention. For those meta-analyses, we extracted information on the following: pharmacological intervention(s) assessed, common outcome(s) evaluated, type of studies included as data sources, and statistical analysis method used. The results of meta-analysis assessing the same clinical question were described and, if different, in which direction. Differences in the choice of the meta-analytic method between meta-analyses, which evaluated the same clinical question, were presented.

The pharmacological interventions were coded according to Anatomical Therapeutic Chemical (ATC) classification system, second-level therapeutic subgroup (WHO COLLABORATION CENTRE FOR DRUG STATISTICS METHODOLOGY, 2014). The AEs, which were established as meta-analyses' primary outcomes were classified according to the Medical Dictionary for Regulatory Activities (MedDRA), high-level group term (HLGT) (MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES, 2011).

II.4. RESULTS

The initial search yielded 459 citations. Twenty-one articles were excluded because they were not meta-analyses, resulting in 438 full papers identified as "possibly eligible" for inclusion. After reviewing the full publication, 60 meta-analyses were included in the study (Figure II.1) (Supplemental Data II.2).

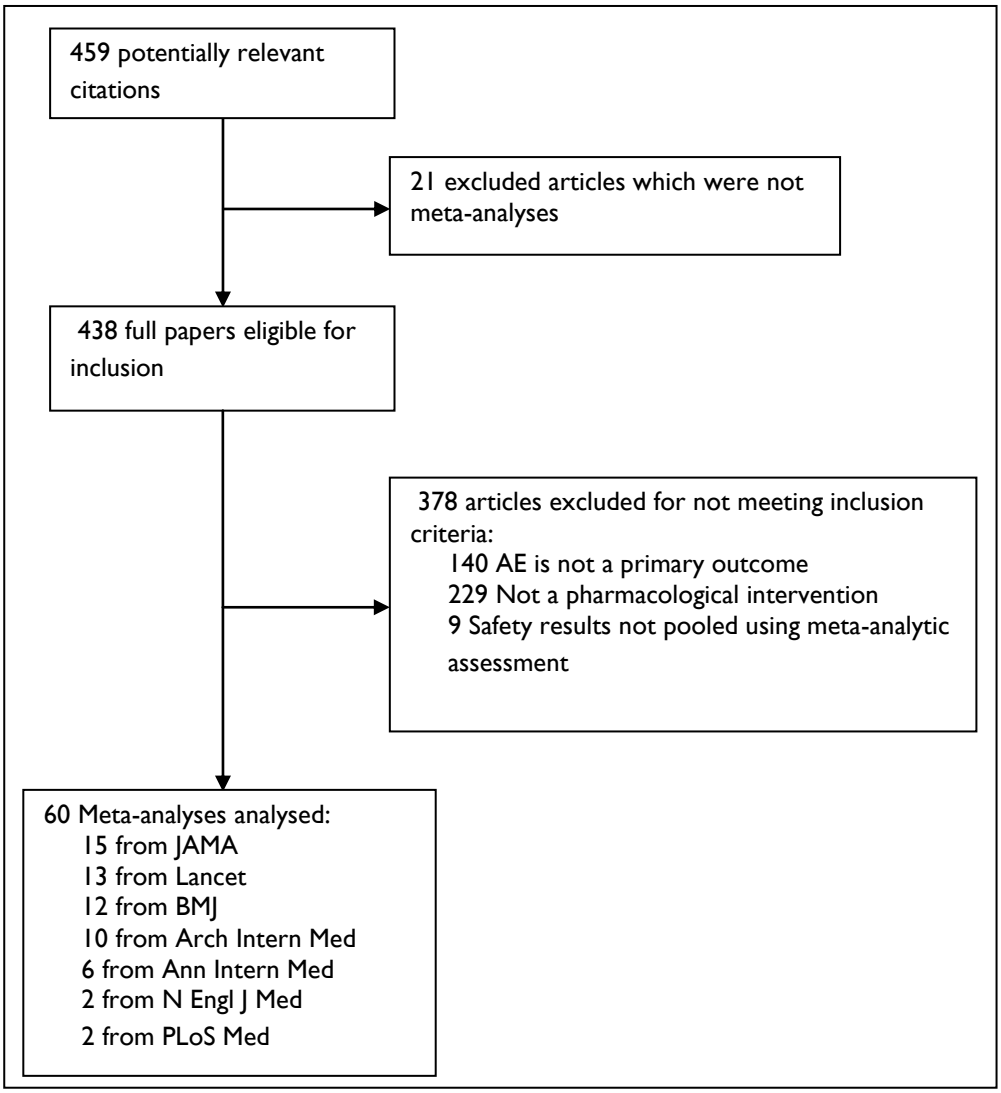


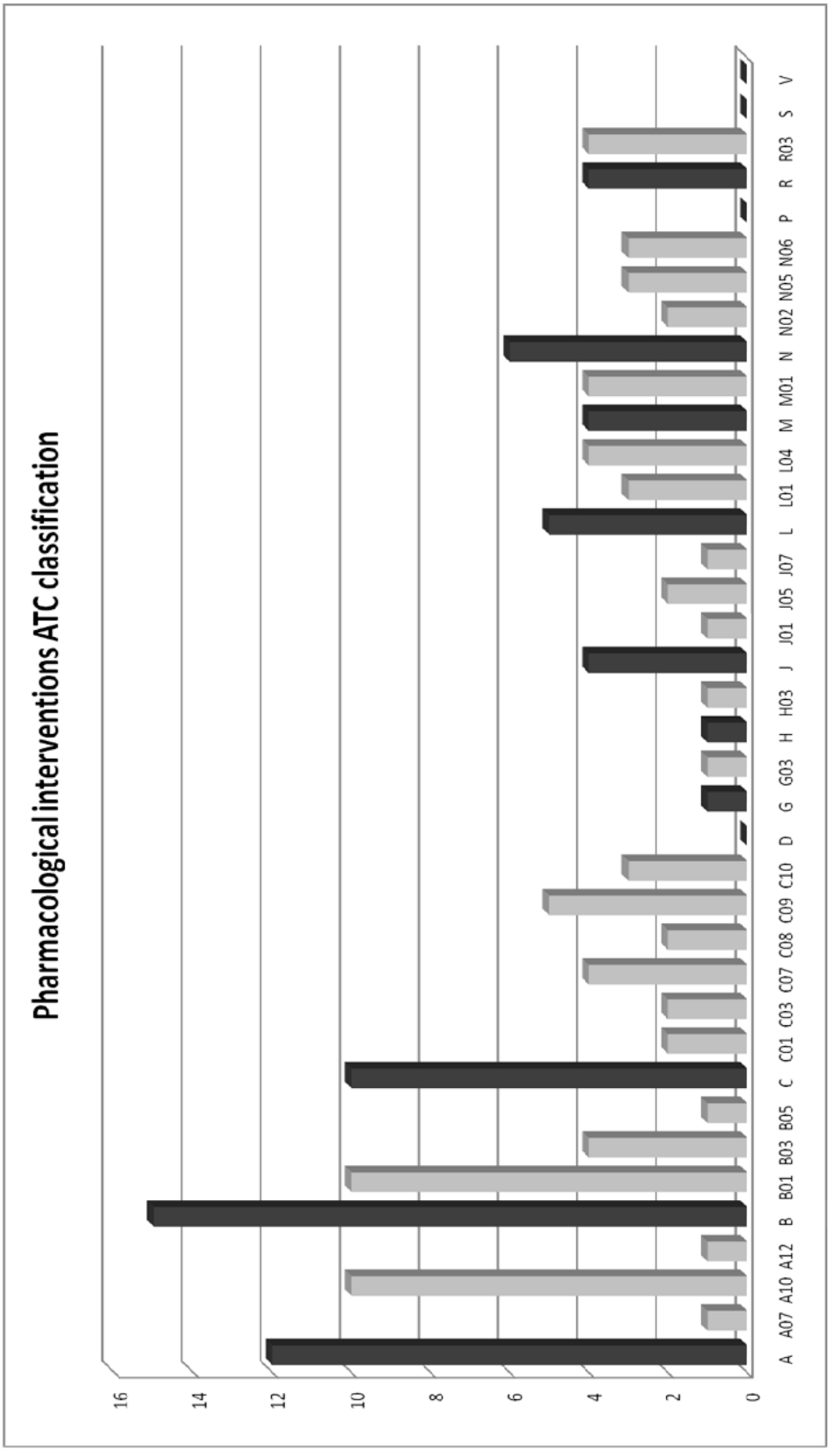
Figure II.1 - Flow diagram of identification of meta-analyses for inclusion.

Of the 60 included meta-analyses, 15 (25%) were published in the JAMA, 13 (22%) in The Lancet, 12 (20%) in the BMJ, 10 (17%) in the Archives of Internal Medicine, 6 (10%) in the Annals of Internal Medicine, 2 (3%) in the New England Journal of Medicine, and another

2 (3%) in the PLoS Medicine. We did not identify any meta-analysis published in the Annual Review of Medicine throughout the studied period.

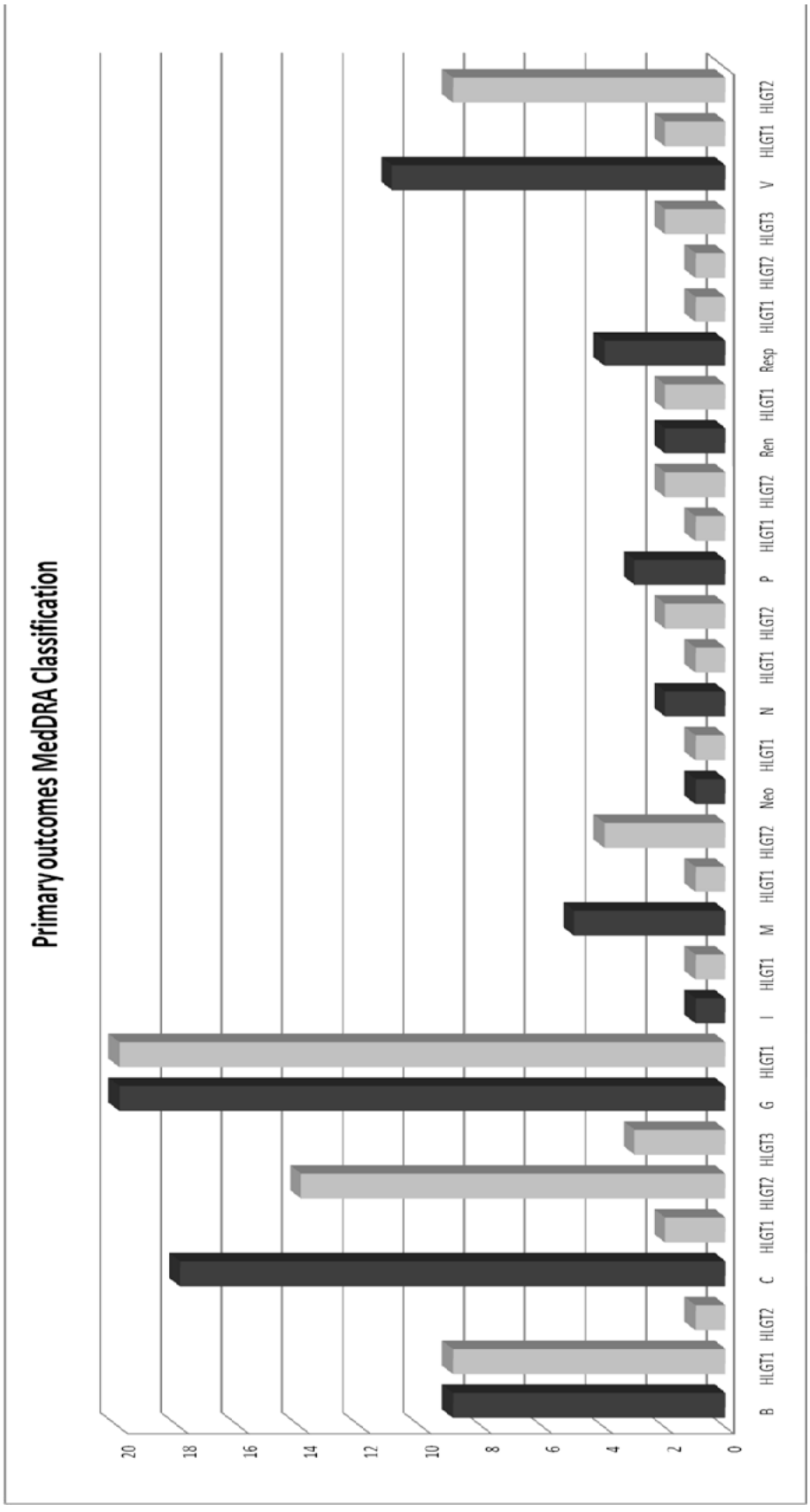
Meta-analyses were mainly directed toward evaluation of pre-specified adverse outcomes of interest (n=48; 80%). Twelve (20%) meta-analyses assessed the whole spectrum of AEs. Of the 60 included studies, 3 (5%) assessed the AEs associated with medical devices eluting drugs. The pharmacological intervention classification according to therapeutic subgroup of the ATC code is presented in Figure II.2. The most frequently assessed pharmacological interventions were "antithrombotic agents" (n=10) and "drugs used in diabetes" (n=10). The AEs of antihypertensive agents, such as "agents acting on the renin-angiotensin system" (n=5), "beta blocking agents" (n=4), "calcium channel blockers" (n=2), and "diuretics" (n=2), also were assessed. One meta-analysis simultaneously assessed AEs of three different pharmacological interventions.

The classification of pre-specified AEs (primary outcome) established according to HLGT MedDRA dictionary is presented in Figure II.3 (48 meta-analyses). The most frequently evaluated AE was "fatal outcomes" (n=20), such as all-cause death outcomes. "Coronary artery disorders", such as myocardial infarction, were the second most frequently evaluated AE (n=14). Risk for occurrence of "coagulopathies and bleeding diathesis (excl thrombocytopenic)", such as bleeding events, and the "embolism and thrombosis", such as stroke or venous thromboembolism, were evaluated by nine meta-analyses. Some meta-analyses evaluated more than one specific AE as primary outcome.



Number of pharmacological interventions according to therapeutic subgroup ATC classification. A - Alimentary tract and metabolism; A07 - Antidiarrheals, intestinal antiinflammatory/antiinfective agents; A10 - Drugs used in diabetes; A12 - Mineral supplements; B - Blood and blood forming organs; B01 - Antithrombotic agents; B03 - Antianemic preparations; B05 - Blood substitutes and perfusion solutions; C - Cardiovascular system; C01 - Cardiac therapy; C03 - Diuretics; C07 - Beta blocking agents; C08 - Calcium channel blockers; C09 - Agents acting on the renin-angiotensin system; C10 - Lipid modifying agents; D - Dermatologicals; G - Genito urinary system and sex hormones; G03 - Sex hormones and modulators of the genital system; H - Systemic hormonal preparations, excluding sex hormones and insulins; H03 - Thyroid therapy; J - Antiinfectives for systemic use; J01 - Antibacterials for systemi0c use; J05 - Antivirals for systemic use; J07 - Vaccines; L - Antineoplastic and immunomodulating agent; L01 - Antineoplastic agents; L04 - Immunosuppressants; M - Musculo-skeletal system; M01 - Antiinflammatory and antirheumatic products; N - Nervous system; N02 - Analgesics; N05 - Psycholeptics; N06 - Psychoanaleptics; P - Antiparasitic products, insecticides and repellents; R - Respiratory system; R03 - Drugs for obstructive airway diseases; S - Sensory organs; V – Various. Some meta-analyses evaluated more than one pharmacological intervention.

Figure II.2 - Pharmacological interventions classification according to therapeutic subgroup (2nd level) of the ATC code.



Number of primary outcome' adverse events classified according to the MedDRA dictionary. B - Blood and lymphatic system disorders; HLGT1 - Coagulopathies and bleeding diathesis (excl thrombocytopenic); HLGT2 - Platelet disorders; C – Cardiac disorders; HLGT1 - Cardiac arrhythmias; HLGT2 - Coronary artery disorders; HLGT3 - Heart failures; G - General disorders and administration site conditions; HLTG1 - Fatal outcomes; I - Infections and infestations; HLGT1 - Infections - pathogen unspecified; M - Metabolism and nutrition disorders; HLGT1 - Electrolyte and fluid balance conditions; HLTG2 - Glucose metabolism disorders (incl diabetes mellitus) Neo - Neoplasms benigh, malignant and unspecified (incl cysts and polyps); HLGT1 - Miscellaneous and site unspecified neoplasms malignant and unspecified; N - Nervous system disorders; HLGT1 - Mental impairment disorders; HLGT2 - Neurological disorders NEC; P - Psychiatric disorders; HLGT1 - Deliria (incl confusion); HLGT2 - Suicidal and self-injurious behaviours NEC; Ren – Renal and urinary disorders; HLGT1 - Renal disorders (excl nephropathies); Resp – Respiratory, thoracic and mediastinal disorders; HLGT1 - Bronchial disorders NEC; HLGT2 - Respiratory disorders NEC; HLGT3 - Respiratory tract infections; V – Vascular disorders; HLGT1 - Decreased and nonspecific blood pressure disorders and shock; HLGT2 - Embolism and thrombosis. HLGT – "High Level Group Term". Some meta-analyses evaluated more than one specific AE as a primary outcome.

Figure II.3 - Meta-analyses adverse events' primary outcome(s) classification according to the MedDRA dictionary, High Level Group Term (HLGT).

II.4.1. EVALUATION OF THE SAME CLINICAL QUESTIONS

Some meta-analyses addressed the same clinical question. In Table II.1, research questions and studied interventions are presented. According to the results, nine clinical questions were evaluated by more than one meta-analysis. All but one meta-analysis included RCTs. Four meta-analysis studied cardiovascular risk associated with rosiglitazone (all RCTs), two studied major bleeding associated with antithrombotic agents (all RCTs), four studied death from all causes associated with erythropoiesis-stimulating agents (all RCTs), three studied death from all causes associated with drug-eluting stents with sirolimus (all RCTs), two studied myocardial infarction associated with non-steroid anti-inflammatory drugs (on included RCTs and other observational studies) , two studied suicidal behavior associated with antidepressants (all RCTs), and two studied pneumonia associated with inhaled corticosteroids (all RCTs).

II.4.1.1. Statistical meta-analytic methods

We compared the statistical methodology for meta-analyses, which addressed the same clinical question. To prevent biased analysis, the majority of meta-analyses compared the results obtained from two meta-analytic methodologies (random or fixed effects models). Not all the meta-analyses, which evaluated the same clinical question, used the same meta-analytic methods. The meta-analytic methodology choice was discussed in all meta-analyses.

For the majority of the meta-analyses, the choice between a fixed effects model and a random effects model was based on the presence of heterogeneity. When between-study heterogeneity was not observed, the authors used a fixed effects model.

Table II.1 - Same clinical questions evaluated by different meta-analyses.

Study	Clinical question	Intervention	Common outcome evaluated	Studies included	Statistical analysis
		A10 – Drugs used in diabetes			
NISSEN and WOLSKI, 2007[29]	"...assess the effects of rosiglitazone on cardiovascular outcomes..."	Rosiglitazone	Myocardial infarction; cardiovascular mortality; all-cause death	RCTs	Peto OR
SINGH *et al*, 2007[7]	"...review the long-term cardiovascular risks of rosiglitazone..."	Rosiglitazone	Myocardial infarction; cardiovascular mortality	RCTs	Fixed-effects RR; Random-effects RR
LAGO *et al*, 2007[23]	"...examine the risk of congestive heart failure and of cardiac death in patients given TZDs."	Pioglitazone; Rosiglitazone	Cardiovascular mortality	RCTs	Fixed-effects RR; Random-effects RR
NISSEN and WOLSKI, 2010[31]	"...review the effects of rosiglitazone therapy on MI and mortality (CV and all-cause)"	Rosiglitazone	Myocardial infarction; cardiovascular mortality; all-cause death	RCTs	Peto OR; Fixed-effects OR
		B01 – Antithrombotic agents			
COOPER *et al*, 2006[38]	"...identify different stroke prevention treatments for atrial fibrillation assessed in randomized controlled trials and to compare them within a single evidence synthesis framework."	Warfarin; Ximelagatran; Indobufen; Triflusal; Acenocoumarol Aspirin;	Major bleedings	RCTs	Random-effects Poisson regression RR
HART *et al*, 2007[43]	"To characterize the efficacy and safety of antithrombotic agents for stroke prevention in patients who have atrial fibrillation..."	Warfarin; Aspirin; Dipyridamole	Major bleedings	RCTs	Random-effects RRR and ARR
		B03 – Antianemic preparations			
BENNET *et al*, 2008[6]	"To evaluate VTE and mortality rates associated with ESA administration for the treatment of anemia among patients with cancer."	Erythropoietin; Darbepoetin.	All-cause death	RCTs	Random-effects HR and RR
BOHLIUS *et al*, 2009[21]	"...to examine the effects of these drugs on the survival of patients with cancer and to identify factors that might modify such effects."	Erythropoietin-alfa; Erythropoietin-beta; Darbepoetin.	All-cause death	RCTs	Fixed-effects meta-regression analysis HR; Random-effects meta-regression analysis HR
PHROMMINTIKUL *et al*, 2007[25]	"...to determine whether targeting different haemoglobin concentrations when treating anaemic patients with chronic kidney disease with erythropoiesis-stimulating agents is associated with altered all-cause mortality and cardiovascular events."	Erythropoietin-alfa; Erythropoietin-beta; Darbepoetin.	All-cause death	RCTs	Fixed-effects RR; Random-effects RR
PALMER *et al*, 2010[41]	"To summarize the effects of ESA treatment on clinical outcomes in patients with anemia and CKD."	Erythropoietin-alfa; Erythropoietin-beta; Darbepoetin.	All-cause death	RCTs	Random-effects RR; Random-effects meta-regression analysis
		L01 – Antineoplastic agents & L04 – Immunosuppressants			
KASTRATI *et al*,	"...to assess the long-term	Sirolimus-eluting	All-cause death	RCTs	Random-

2007[30]	outcome after implantation of sirolimus-eluting stent..."	stents; Bare-metal stents.			effects HR; Random-effects meta-regression analysis
STETTLER *et al*, 2007[24]	"...to compare the safety and effectiveness of these stents." (drug-eluting stents vs. bare-metal stents or drug-eluting stents heat-to-head)	Paclitaxel-eluting stents; Sirolimus-stents; Bare-metal stents.	All-cause death	RCTs	Multivariable Bayesian hierarchial random-effects HR; Random-effects HR
STETTLER *et al*, 2008[50]	"To compare the effectiveness and the safety of three types of stents (sirolimus eluting, paclitaxel eluting, and bare metal) in people with or without diabetes mellitus."	Paclitaxel-eluting stents; Sirolimus-stents; Bare-metal stents.	All-cause death	RCTs	Multivariable Bayesian hierarchial random-effects HR; Random-effects Poisson regression model HR
		M01 – Antiinflammatory and antirheumatic products			
KEARNEY *et al*, 2006[56]	"To assess the effects of selective cyclo-oxygenase-2 (COX 2) inhibitors and traditional non-steroidal anti-inflammatory drugs (NSAIDs) on the risk of vascular events."	Rofecoxib; Celecoxib; Etoricoxib; Lumiracoxib; Valdecoxib; Ibuprofen; Diclofenac; Naproxen.	Myocardial infarction	RCTs	Peto "one step" approximation RR (rate ratio)
MCGETTIGAN *et al*, 2006[12]	"…to compare the risks of serious cardiovascular events with individual NSAIDs and cyclooxygenase 2 inhibitors."	Rofecoxib; Celecoxib; Meloxicam; Ibuprofen; Diclofenac; Naproxen; Indomethacin; Piroxicam.	Myocardial infarction	Case-control studies; Cohort studies.	Random-effects RR
		N06 – Psychoanaleptics			
BRIDGE *et al*, 2007[10]	"To assess the efficacy and risk of reported suicidal ideation/suicide attempt of antidepressants for treatment of pediatric major depressive disorder (MDD), obsessive-compulsive disorder (OCD), and non-OCD anxiety disorders."	Fluoxetine; Paroxetine; Sertraline; Citalopram; Escitalopram; Venlafaxine; Nefazodone; Mirtazipine.	Suicide ideation; Suicide attempt; Suicide preparatory actions; Suicide ideation/Suicide attempt.	RCTs	Random-effects RD
STONE *et al*, 2009[49]	"To examine the risk of suicidal behaviour within clinical trials of antidepressants in adults."	Fluoxetine; Fluvoxamine; Duloxetine; Paroxetine; Sertraline; Citalopram; Escitalopram; Venlafaxine; Nefazodone; Mirtazipine; Bupropion; Amitriptyline; Clomipramine; Desipramine; Dosulepin; Imipramine; Trazodone; Mianserin	Suicide ideation; Suicide attempt; Suicide preparatory actions.	RCTs	Random-effects logistic regression OR and RD

R03 – Drugs for obstructive arway diseases					
DRUMMOND *et al*, 2008[2]	"...synthesize the effects of inhaled corticosteroid therapy on mortality and adverse events in patients with stable chronic obstructive pulmonary disease."	Fluticasone; Budesonide;	Any pneumonia	RCTs	Random-effects RR Fixed-effects RR
SINGH *et al*, 2009[33]	"...to ascertain the risk of pneumonia with long-term inhaled corticosteroid use among patients with chronic obstructive pulmonary disease."	Fluticasone; Budesonide; Beclomethasone.	Any pneumonia	RCTs	Random-effects RR Fixed-effects RR

The references of the studies included in this table are presented at Supplemental Data 2. RCT – randomized clinical trial; RR – relative risk; OR – odds ratio; HR – hazard ratio

II.4.2. DATA SOURCES AND SEARCH STRATEGY

Nearly all the meta-analyses (n=57; 95%) searched electronic databases (median 3; range, 1-7), with a total of 19 different electronic databases reported (Table II.2). MEDLINE was the electronic database most frequently searched in meta-analyses (n=51/57; 89%), followed by Cochrane Library (n=47/57; 82%), and EMBASE (n=37/57; 65%). One meta-analysis did not report which electronic databases were searched. Of the 57 meta-analyses that searched electronic databases, 13 (23%) meta-analyses completely specified the search strategy. Twenty-four meta-analyses (42%) reported the use of medical subject headings in their search strategies.

Forty-nine (82%) meta-analyses searched at least one additional data source, besides electronic databases, such as retrieved trials (n=34/49; 70%) or references from reviews (n=24/49; 49%). Other additional data sources were the contact with the manufacturer company (n=21/49; 43%), data from scientific societies meetings (n=17/49; 35%) and contact with the regulatory agencies (n=17/49; 35%), and relevant websites, such as clinicaltrials.gov (n=14/49; 29%).

Table II.2 - Data sources and search strategy of the meta-analyses studied.

	***n* = 60**	
Data sources searched		
Reported at least one source	**60**	**100%**
Electronic database	**57**	**95%**
MEDLINE	51	89%
Cochrane Library	47	82%
EMBASE	37	65%
PubMed	7	12%
Web of Science	6	11%
CINAHL	3	5%
OVID	2	4%
HealthSTAR	1	2%
EBM	1	2%
CancerLit	1	2%
SIGLE	1	2%
PsycINFO	1	2%
PsychLit	1	2%
Cum. Index to Nursing	1	2%
Int. Pharm. Abstracts	1	2%
All. Compl. Med	1	2%
Google Scholar	1	2%
CRISP	1	2%
German Inst. Med. Doc. Inf.	1	2%
Not reported	1	2%
Searched additional data source	**49**	**82%**
References from retrieved trials	34	70%
References from reviews	24	49%
Contact with manufacturer company	21	43%
Scientific society meetings	17	35%
Regulatory agencies	17	35%
Relevant websites	14	29%
Contact with trial investigator	12	24%
Contact with experts	11	22%
Scientific journal in the area	3	6%
Book chapters	2	4%
Product information sheet	1	2%
Helath organizations	1	2%

Abbreviations: *Cum. Index to Nursing* - Cumulative Index to Nursing and Allied Health Literature; *Int. Pharm. Abstracts* – International Pharmaceutical Abstracts; *All. Compl. Med* – Allied and Complementary Medicine; *German Inst. Med. Doc. Inf.* – German Institute of Medical Documentation and Information

II.4.3. TYPE OF STUDIES INCLUDED IN META-ANALYSIS AND ANALYSIS OF RESULTS

Of the meta-analyses included in this study, 25 (42%) assessed both efficacy and safety outcomes, whereas 35 (58%) assessed only safety outcomes (Table II.3). Twenty-four (96%) of the meta-analyses assessing efficacy and safety included only experimental studies (RCTs), and 1 (4%) meta-analysis included both experimental and observational studies (RCTs, non-RCTs, prospective cohort, retrospective cohort, case-control, and cross-sectional studies).

Table II.3 - Meta-analyses design, type of included studies and their publication status, and assessment of publication bias.

	n=60	
Type of study design		
Efficacy and Safety outcomes	**25**	**42%**
Experimental studies	24	96%
Observational studies	-	
Experimental and Observational studies	1	4%
Safety outcomes	**54**	**58%**
Experimental studies	29	83%
Observational studies	2	6%
Experimental and Observational studies	3	9%
Study design not reported	1	3%
Publication status of included studies		
Published	31	52%
Published and Unpublished	21	35%
Published and Short communications	8	13%
Publication Bias		
Assessed	25	42%
Not assessed	35	58%

Of the meta-analyses that assessed safety outcomes, 29 (83%) included only experimental studies (RCTs), 3 (9%) included both experimental and observational studies, 2 (6%) included only observational studies, and 1 (3%) did not report the design of the studies. Of the three meta-analyses that comprised experimental and observational studies, one included RCTs, *post hoc* analysis within an RCT, and prospective and retrospective cohort studies; 1 included RCTs and prospective and retrospective cohort studies; and 1 included RCTs, prospective cohort, and case-control studies. Of the 2 meta-analyses that comprised

only observational studies, one included prospective cohort and case-control studies, and the other one included prospective cohort, case-control, and cross-sectional studies.

According to the publication status, 31 (52%) meta-analyses included only published studies, 21 (35%) included published and unpublished studies, and 8 (13 %) meta-analyses included published studies and short communications (Table II.3).

Less than half (n=25; 42%) of the meta-analyses reported publication bias assessment.

II.4.3.1. Meta-analyses which included different types of studies

Of the four meta-analyses that comprised experimental and observational studies, we evaluated if the pooled results were different and, if so, in which direction. Two meta-analyses compared the results pooled from RCTs with the results pooled from observational studies. Of these, in one meta-analysis, the RCTs' pooled OR of harms overlapped the OR pooled from observational studies. In the other, for one safety outcome (discontinuation of the drug due to AEs, mainly because of cough) the observational studies' pooled OR was beyond chance comparing with RCTs' pooled OR.

Two meta-analyses did not compare the results pooled from RCTs with the results pooled from observational studies. In one of these, observational studies provided information on safety outcomes, which were not reported in RCTs.

II.4.4. QUALITY ASSESSMENT OF THE STUDIES INCLUDED IN META-ANALYSES

Forty-one (68%) meta-analyses reported the quality assessment of the included studies, of which, 30 (73%) were based on published quality assessment instruments (Table II.4). Twelve different instruments were used to assess methodological quality of RCTs. Of these, Jadad scale (n=10; 24%) and the Cochrane Handbook f or Systematic Reviews of Interventions (n=6; 15%) were the most frequently used (JADAD *et al.*, 1996; HIGGINS and GREEN, 2011). Four meta-analyses assessed the quality of observational studies included, two using the method of Downs and Black (n=2; 5%), one using the Newcastle-Ottawa Scale (n=1; 2%), and the other based its assessment on MOOSE guidelines (n=1; 2%) (DOWNS and BLACK, 1998; WELLS *et al.*, 2011; STROUP *et al.*, 2000). Five meta-analyses assessed the methodological quality of the included studies according to more than one quality assessment instrument. The remaining 11 (27%) meta-analyses did not report the use of

instruments to assess the methodological quality of the included studies. Instead, they described the indicators used for quality assessment.

Table II.4 - Quality assessment of the studies included in meta-analyses.

	n=60	
Assessed methodological quality	**41**	**68%**
Based on existing instruments	30	73%
Jadad scale	10	24%
Coch. Hand. For Syst. Rev. Interventions	6	15%
QUOROM	3	7%
Juni et al, 2001	3	7%
Schulz et al, 1995	3	7%
Meade and Richardson, 2007	2	5%
Moher et al, 1998	1	2%
US Preventive Task Force	1	2%
NHS Centre for Reviews and Dissemination	1	2%
CONSORT	1	2%
Schulz, KF	1	2%
Altman and Schulz, 2001	1	2%
Detsky et al, 1992	1	2%
Downs and Black, 1998	2	5%
Newcastle-Ottawa Scale	1	2%
MOOSE	1	2%
Not based on existing instruments	11	27%
Not assessed	**19**	**32%**

Of the 41 meta-analyses that assessed the methodological quality of the studies, 35 (85%) reported the results of this assessment. Nine (22%) meta-analyses evaluated the effect of the different quality scored studies on the results, by performing a sensitivity analysis. The methodological quality assessment was used as inclusion criterion in seven (17%) meta-analyses, of which, three used the Cochrane Handbook for Systematic reviews of Interventions to assess the quality reporting of AEs reported (LOKE, PRICE and HERXHEIMER, 2011).

II.4.5. GUIDELINES DESCRIBED AS USED IN THE ELABORATION OF META-ANALYSES

In 11 (18%) meta-analyses, the authors said they used established recommended statements to elaborate meta-analyses: seven (64%) according to QUOROM guidelines; two

(18%) using the Cochrane Handbook for Systematic reviews of Interventions guidelines; and PRISMA, MOOSE, and Agency for Healthcare Research and Quality guidelines were used to report one meta-analysis each (Table II.5) (HIGGINS and GREEN, 2011; STROUP *et al.*, 2000; MOHER *et al.*, 1999; MOHER *et al.*, 2009; MATCHAR *et al.*, 2007). Of the 11 meta-analyses, one was reported according to both QUOROM and Cochrane Handbook guidelines.

Table II.5 - Reporting guidelines used by meta-analyses.

	n=60	
Reported using existing guidelines	**11**	**18%**
QUOROM	7	64%
Coch. Hand. For Syst. Rev. Interventions	2	18%
PRISMA	1	9%
AHRQ	1	9%
MOOSE	1	9%
Use not reported	**49**	**82%**

II.5. DISCUSSION

This study provides evidence on the use of meta-analysis to evaluate the safety of pharmacological interventions published in eight medical journals from 2005 to 2010, selected according to their impact factor on the area of general/internal medicine.

We identified 438 meta-analyses published during the studied period, of which, 60 (14%) assessed drug safety as a primary outcome. The majority was designed to assess drug efficacy and to evaluate non-pharmacological interventions. This result supports our previous concerns that only a limited number of published meta-analyses are currently devoted to monitor safety profile of pharmacological interventions. We also found that several meta-analyses evaluated the same clinical question during the studied period (e.g., risk of cardiovascular events associated with rosiglitazone therapy). This was expected because, during the studied period, some pharmacological interventions were under evaluation because of doubts about their safety profile.

The 60 meta-analyses assessing AEs included experimental studies, observational studies, and both simultaneously. Experimental studies were the predominant source of information in this sample. Experimental studies are accepted to be the gold standard to evaluate drug efficacy, mainly because randomized allocation protects against bias and confounding effects that can threaten the validity of the study. Experimental designs to evaluate drug safety are difficult to carry out, often because of ethical reasons. However, experimental designs that evaluate drug efficacy use rigorous criteria for patient selection and use efficacy end points to estimate sample size and follow-up time. Thus, data on frequent and acute AEs may be observed in these studies, but unknown, rare, and/or long-term latency AEs are difficult to be identified.

Most evidence on harms is more likely to be obtained from pharmacovigilance activities, particularly by using observational studies. Although these studies are more prone to bias and confounding, they offer the advantage of a naturalistic observation, more likely to include a broad representation of the population at risk. However, as it can be found in the present study, meta-analyses including observational studies aiming at drug safety monitoring are relatively few, although there are a vast number of observational studies published, some with remarkable contributions on the study of drug safety (LACROIX *et al.*, 2003; TROMP *et al.*, 2001; VAN STAA, LEUFKENS and COOPER, 2001; SCOLNIK *et al.*, 1994).

The assessment of the methodological quality was reported for the majority of the studies included in the identified meta-analyses. However, for the purpose of quality

assessments, different instruments have been developed and applied, giving place for heterogeneity in such evaluations. The use of different instruments can lead to different considerations in terms of weight assigned to key domains. In our study, different scores from quality assessments were not weighted on the overall results in several meta-analyses, therefore not reflecting the impact of poor quality studies on the presented findings. Although it is widely recommended that epidemiologic studies undergo some type of quality review, the method of assessing and incorporating the quality scores is a matter of ongoing debate (JÜNI *et al*., 1999). Assessing the influence of specific relevant methodological aspects on effects sizes has been a preferred approach recently (JÜNI *et al*., 1999).

Few meta-analyses included unpublished studies and assessed publication bias. This has been documented as an essential effort in meta-analyses studies because it can influence the accuracy of data and lead to misleading results. The potential consequences of publication bias have been debated for some time (STERNE, EGGER and SMITH, 2001; BAX and MOONS, 2010).

The choice of the selected journals probably shaped what we found because numerous other journals devoted to specific clinical areas or covering the disciplines of pharmacoepidemiology and pharmacovigilance have published relevant meta-analyses, some of those including different studies designs as data sources (HENNESSY *et al.*, 2001; GAGNE, GRIESDALE and SCHNEEWEISS, 2009; LOKE, JEEVANANTHAM and SINGH, 2009; TOH and HERNÁNDEZ-DÍAZ, 2007; CHEN and ASHCROFT, 2006). These journals were selected because they are the most widely read and quoted and, thus, are the most likely to influence clinical practice. Safety is a matter of concern in the use of medicines. To identify if the selected journals highlight safety in the same extent that efficacy is expected to be highlighted was a selected objective of this study. Although this fact may be considered a limitation, its intention was to explore the possibility of imbalance in equal terms of safety and efficacy, therefore modulating clinicians' judgments toward efficacy. The present study only included published meta-analyses. Therefore, eventually existing non-published relevant meta-analyses are not represented.

Despite that we had found meta-analyses devoted to evaluate the safety of pharmacological interventions, they included mostly experimental studies. In 60 meta-analyses, 6 (10%) included observational studies. The usefulness of the observational studies is the detection of rare and long-term latency AEs and the study of minority populations. The evaluation of rare AEs is recognized to be challenging (BRADBURN *et al.*, 2007). The

inclusion of different study designs in meta-analyses should be properly planned, depending on the type of outcome, which is being evaluated.

The Safety Planning, Evaluation and Reporting Team formed in 2006 recommend that sponsors develop a program safety analysis plan beginning with first clinical studies, as a tool to proactively plan for meta-analyses at regular intervals during marketed use of a product (CROWE *et al.*, 2009). The ICH E9 guideline also states that meta-analyses should be prospectively planned with the RCTs program in the development of a new treatment (INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE, 1998).

Meta-analysis is recognized as a valuable method to address safety questions, either defined prospectively or those that are retrospectively addressed. However, the combination of information across all studies is facilitated by the use of consistent approaches in the definitions, data collection, processing, and analysis during drug development and postmarketing (IOANNIDIS *et al.*, 2004; BEHRMAN *et al.*, 2011). The role of meta-analysis in pharmacovigilance is a matter of ongoing debate, and efforts are being made to develop guidelines on the use of meta-analysis in drug safety assessments (DRUG INFORMATION ASSOCIATION, 2011; THE COCHRANE COLLABORATION, 2014).

II.6. BIBLIOGRAPHY

AKICI, A.; OKTA, S. – Rational pharmacotherapy and pharmacovigilance. **Curr Drug Saf.** 2;1 (2007) 65-69.

BATES, D.W. – Incidence of adverse drug events and potential adverse drug events. Implications for prevention. **JAMA**. 274;1 (1995) 29-34.

BAX, L.; MOONS, K.G. – Beyond publication bias. **J Clin Epidemiol.** 64;5 (2010) 459-462.

BEHRMAN, R.E. [et al] – Developing the Sentinel System - A National Resource for Evidence Development. **N Engl J Med.** 364;6 (2011) 498-499.

BRADBURN, M.J. [et al] – Much ado about nothing: a comparison of the performance of meta-analytical methods with rare events. **Stat Med.** 26;1 (2007) 53-77.

CANONICO, M. [et al] – Hormone replacement therapy and risk of venous thromboembolism in postmenopausal women: systematic review and meta-analysis. **BMJ**. 336;7655 (2008) 1227-1231.

CHEN, L.C.; ASHCROFT, D.M. – Do selective COX-2 inhibitors increase the risk of cerebrovascular events? A meta-analysis of randomized controlled trials. **J Clin Pharm Ther.** 31;6 (2006) 565-576.

CROWE, B.J. [et al] – Recommendations for safety planning, data collection, evaluation and reporting during drug, biologic and vaccine development: a report of the safety planning, evaluation, and reporting team. **Clin Trials.** 6;5 (2009) 430-440.

DERSIMONIAN, R.; LAIRD, N. – Meta-analysis in clinical trials. **Control Clin Trials.** 7;3 (1986) 177-188.

DOWNS, S.H.; BLACK, N. – The feasibility of creating a checklist for assessment of the methodological quality of both the randomised and nonrandomised studies of health care interventions. **J Epidemiol Community Health.** 52;6 (1998) 377-384.

DRUG INFORMATION ASSOCIATION – DIA/FDA Best practices for regulatory information synthesis of randomized controlled trials for product safety evaluation. Drug Information Association, Inc. (2011) Available from http://www.diahome.org/DIAHome/resources/content.aspx?type=eopdf&file=%2fproductfiles%2f25159%2f11011%2Epdf.

EUROPEAN MEDICINES AGENCY – Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. (CPMP/ICH/377/95) Committee for Proprietary Medicinal Products (CPMP), 1995.

GAGNE, J.J.; GRIESDALE, D.E.G.; SCHNEEWEISS, S. – Aprotin and the risk of death and renal dysfunction in patients undergoing cardiac surgery: a meta-analysis of epidemiologic studies. **Pharmacoepidemiol Drug Saf.** 18;4 (2009) 259-268.

HENNESSY, S. [et al] – Risk of venous thromboembolism form oral contraceptives containing gestodene and desogestrel versus levonogestrel: a meta-analysis and formal sensitivity analysis. **Contraception**. 64;2 (2001) 125-133.

HICKS, L.K. [et al] – A meta-analysis and systematic review of thalidomide for patients with previously untreated multiple myeloma. **Cancer Treat Rev.** 2008;34(5):442-452.

HIGGINS, J.P.T.; GREEN, S. – Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration, 2011. (2011) Available from www.cochrane-handbook.org.

HOPEWELL, S.; WOLFENDEN, L.; CLARKE, M. – Reporting of adverse events in systematic reviews can be improved: survey results. **J Clin Epidemiol.** 61;6 (2008) 597-602.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE – Statistical principles for clinical trials: E9, 1998.

IOANNIDIS, J.P. [et al] – Better reporting of harms in randomized trials: an extension of the CONSORT statement. **Ann Intern Med.** 141;10 (2004) 781-788.

IOANNIDIS, J.P.A.; LAU, J. – Completeness of safety reporting in randomized trials: an evaluation of 7 medical areas. **JAMA.** 285;4 (2001) 437-443.

JADAD, A.R. [et al] – Assessing the quality of reports of randomized clinical trials: is blinding necessary? **Control Clin Trials.** 17;1 (1996) 1-12.

JÜNI, P. [et al] – The hazards of scoring the quality of clinical trials for meta-analysis. **JAMA.** 282;11 (1999) 1054-1060.

KIM, C.J.; BERLIN, J.A. – The use of meta-analysis in pharmacoepidemiology**.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 353-366.

LACROIX, A.Z. [et al] – Statin use, clinical fracture, and bone density in postmenopausal women: results from the Women's Health Initiative Observational Study. **Ann Intern Med.** 139;2 (2003) 97-104.

LOKE, Y.K.; DERRY, S.; ARONSON, J.K. – A comparison of three different sources of data in assessing the frequencies of adverse reactions to amiodarone. **Br J Clin Pharmacol.** 57;5 (2004) 616-621.

LOKE, Y.K.; JEEVANANTHAM, V.; SINGH, S. – Biphosphonates and atrial fibrillation: systematic review and meta-analysis. **Drug Saf.** 32;3 (2009) 219-228.

LOKE, Y.K.; PRICE, D.; HERXHEIMER, A. – Adverse effects. In: HIGGINS, Jullian, GREEN, Sally, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration, 2011. (2011) Available from www.cochrane-handbook.org.

MADRE, L. [et al] – Views from Academia, Industry, and Regulatory Agencies. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 63-88.

MATCHAR, D.B. [et al] – Comparitive Effectiveness of Angiotensin Converting Enzyme Inhibitors (ACEIs) and Angiotensin II Receptor Antagonists (ARBs) for Treating Essential Hypertension. Comparative Effectiveness Review No. 10. Agency for Healthcare Research and Quality. (2007) Available from http://www.effectivehealthcare.ahrq.gov/ehc/products/12/48/ACEI_ARBExecSummary.pdf.

MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES – Support Documentation: MedDRA v14.0. (2011) Available from http://www.meddra.org.

MOHER, D. [et al] – Improving the quality of reports of meta-analysis of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analysis. **Lancet.** 354;9193 (1999) 1896-1900.

MOHER, D. [et al] – Preferred Reporting Items for Systematic Reviews and Meta-Analysis: The PRISMA Statement. **PLoS Med.** 6(7) (2009) e100097. doi: 10.1371/journal.pmed.100097.

PAPANIKOLAOU, P.N.; CHRISTIDI, G.D.; IOANNIDIS, J.P.A. – Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. **CMAJ**. 174;5 (2006) 635-641.

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

SCOLNIK, D. [et al]. – Neurodevelopment of children exposed in utero to phenytoin and carbamazepine monotherapy. **JAMA**. 271;10 (1994) 767-770.

STERNE, J.A.C.; EGGER, M.; SMITH, G.D. – Systematic reviews in health care: investigating and dealing with publication and other biases in meta-analysis. **BMJ**. 323;7304 (2001) 101-105.

STROM, B.L. – **Study designs available from pharmacoepidemiology studies**. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006a, ISBN: 978-0-470-02925-1. p. 13-24.

STROUP, D.F. [et al] – Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. **JAMA**. 283;15 (2000) 2008-2012.

THE COCHRANE COLLABORATION – Methodology Review Group. (2014) Available from http://www.cochrane.org/contact/review-groups.

THOMPSON REUTERS – Science journal citation reports ISI. (2014) Available from http://scientific.thomson.com/products/jcr/

TOH, S.; HERNÁNDEZ-DÍAZ, S. – Statins and fracture risk: a systematic review. **Pharmacoepidemiol Drug Saf.** 16;6 (2007) 627-640.

TROMP, A.M. [et al] – Fall-risk screening test: a prospective study on predictors for falls in community-dwelling elderly. J Clin Epidemiol. 54;8 (2001) 837-844.

VAN STAA, T.P.; LEUFKENS, H.G.; COOPER, C. – Use of inhaled corticosteroids and risk of fractures. **J Bone Miner Res.** 16;3 (2001) 581-588.

VANDENBROUCKE, J.P. – Benefits and harms of drug treatments. **BMJ**. 329;7456 (2004b) 2-3.

VANDENBROUCKE, J.P. – When are observational studies as credible as randomized trials? **Lancet.** 363;9422 (2004a) 1728-1731.

VANDENBROUCKE, J.P.; PSATY, B.M. – Benefits and risks of drug treatments: how best to combine the best evidence on benefits with the best data about adverse effects. **JAMA**. 300;2 (2008) 2417-2419.

WELLS, G. [et al] – The Newcaslte-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis. Ottawa Hospital Research Institute, 2011. (2011) Available from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

WHO COLLABORATION CENTRE FOR DRUG STATISTICS METHODOLOGY – ATC/DDD Index 2014. (2014) Available from http://www.whocc.no/atc_ddd_index/.

II.7. SUPPLEMENTAL DATA II

II.7.1. SUPPLEMENTAL DATA II.1 - SEARCH STRATEGY

Supplemental Table II.1 - Search Strategy.

Search	PubMed	Results
1	"N Engl J Med" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	10
2	"Lancet" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	81
3	"JAMA" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	71
4	"BMJ" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	133
5	"Arch Intern Med" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	59
6	"Ann Intern Med" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	73
7	"PLoS Med" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	32
8	"Annu Rev Med" [journal] Limits: meta-analysis; date range 2005/10/01 – 2010/09/30	0
9	Total	459

II.7.2. SUPPLEMENTAL DATA II.2 - META-ANALYSES' SAMPLE ANALYZED IN THIS STUDY

1. PHUNG, O.J. [et al] – Effect of noninsulin antidiabetic drugs added to metformin therapy on glycemic control, weight gain, and hypoglycaemia in type 2 diabetes. **JAMA**. 202;14 (2010) 1410-1418.

2. DRUMMOND, M.B. [et al] – Inhaled corticosteroids in patients with stable chronic obstructive pulmonary disease: a systematic review and meta-analysis. **JAMA**. 300;20 (2008) 2407-2416.

3. NALLURI, S.R. [et al] – Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. **JAMA**. 300;19 (2008) 2277-2285.

4. SINGH, S.; LOKE, Y.K.; FURBERG, C.D. – Inhaled anticholinergics and risk of major adverse cardiovascular events in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. **JAMA**. 300;12 (2008) 1439-1450.

5. NATANSON, C. [et al] – Cell-free haemoglobin-based blood substitutes and risk of myocardial infarction and death. **JAMA**. 299;19 (2008) 2304-2312.

6. BENNET, C.L. [et al] – Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer associated anemia. **JAMA**. 299;8 (2008) 914-924.

7. SINGH, S.; LOKE, Y.K.; FURBERG, C.D. – Long-term risk of cardiovascular events with rosiglitazone: a metaanalysis. **JAMA**. 298;10 (2007) 1189-1195.

8. LINCOFF, A.M. – Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. **JAMA**. 298;10 (2007) 1180-1188.

9. AMORI, R.E.; LAU, J.; PITTAS, A.G. – Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. **JAMA**. 298;2 (2007) 194-206.

10. BRIDGE, J.A. [et al] – Clinical response and risk for reported suicidal ideation and suicide attempts in pediatric antidepressant treatment: a meta-analysis of randomized controlled trials. **JAMA**. 297;11 (2007) 1241-1252.

11. ZHANG, J.; DING, E.L.; SONG, Y. – Adverse effects of cyclooxygenase 2 inhibitors on renal and arrhythmia events: meta-analysis of randomized trials. **JAMA**. 296;13 (2006) 1619-1632.

12. MCGETTIGAN, P.; HENRY, D. – Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. **JAMA**. 296;13 (2006) 1633-1644.

13. BONGARTZ, T. [et al] – Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. **JAMA**. 295;19 (2006) 2275-2285.

14. BERGER, J.S. [et al] – Aspirin for the primary prevention of cardiovascular events in women and men: a sex-specific meta-analysis of randomized controlled trials. **JAMA**. 295;3 (2006) 306-313.

15. SCHNEIDER, L.S.; DAGERMAN, K.S.; INSEL, P. – Risk of death with atypical antipsychotic drug treatment for dementia: meta-analysis of randomized placebo controlled trials. **JAMA**. 294;15 (2005) 1934-1943.

16. JUN, M. [et al] – Effects of fibrates on cardiovascular outcomes: a systematic review and meta-analysis. **Lancet.** 375;9829 (2010) 1875-1884.

17. SATTAR, N. [et al] – Statins and risk of incident diabetes: a collaborative metaanalysis of randomised statin trials. **Lancet**. 375;9717 (2010) 834-845.

18. SIN, D.D. [et al] – Budesonide and the risk of pneumonia: a meta-analysis of individual patient data. **Lancet.** 374;9691 (2009) 712-719.

19. ANTITHROMBOTIC TRIALISTS' (ATT) COLLABORATION. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. **Lancet.** 373;9678 (2009) 2034-2040.

20. RAY, K.K. [et al] – Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. **Lancet.** 373;9677 (2009) 1765-1772.

21. BOHLIUS, J. [et al] – Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. **Lancet.** 373;9674 (2009) 1532-1542.

22. BANGALORE, S. [et al] – Perioperative beta-blockers in patients having noncardiac surgery: a meta-analysis. **Lancet.** 372;9654 (2008) 1962-1976.

23. LAGO, R.M.; SINGH, P.P.; NESTO, R.W. – Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. **Lancet**. 370;9593 (2007) 1129-1136.

24. STETTLER, C. [et al] – Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. **Lancet**. 370;9591 (2007) 937-948.

25. PHROMMINTIKUL, A. [et al] – Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta-analysis. **Lancet**. 369;9559 (2007) 381-388.

26. ELLIOT, W.J.; MEYER, P.M. – Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. **Lancet**. 369;9557 (2007) 201-207.

27. CHOU, R. [et al] – Initial highly-active antiretroviral therapy with a protease inhibitor versus a non-nucleoside reverse transcriptase inhibitor: discrepancies between direct and indirect meta-analysis. **Lancet**. 368;9546 (2006) 1503-1515.

28. KEELEY, E.C.; BOURA, J.A.; GRINES, C.L. – Comparison of primary and facilitated percutaneous coronary interventions for ST-elevation myocardial infarction: quantitative review of randomised trials. **Lancet**. 367;9510 (2006) 579-588.

29. NISSEN, S.E.; WOLSKI, K. – Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. **N Engl J Med.** 356;24 (2007) 2457-2471.

30. KASTRATI, A. [et al] – Analysis of 14 trials comparing sirolimus-eluting stents with baremetal stents. **N Engl J Med.** 356;10 (2007) 1030-1039.

31. NISSEN, S.E.; WOLSKY, K. – Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. **Arch Intern Med.** 170;4 (2010) 1191-1201.

32. WOOLCOTT, J.C. [et al] – Meta-analysis of the impact of 9 medication classes on falls in elderly persons. Arch Intern Med 169;21 (2009) 1952-1960.

33. SINGH, S.; AMIN, A.V.; LOKE, Y.K. – Long-term use of inhaled corticosteroids and the risk of pneumonia in chronic obstructive pulmonary disease: a meta-analysis. **Arch Intern Med.** 169;3 (2009) 219-229.

34. AKL, E.A. [et al] – Lowmolecular-weight heparin vs unfractionated heparin for perioperative thromboprophylaxis in patients with cancer: a systematic review and meta-analysis. **Arch Intern Med.** 168;12 (2008) 1261-1269.

35. PHILLIPS, C.O. [et al] – Adverse effects of combination angiotensin II receptor blockers plus angiotensin-converting enzyme inhibitors for left ventricular dysfunction: a quantitative review of data from randomized clinical trials. **Arch Intern Med.** 167;18 (2007) 1930-1936.

36. WEIN, L. [et al] – Pharmacological venous thromboembolism prophylaxis in hospitalized medical patients: a meta-analysis of randomized controlled trials. **Arch Intern Med.** 167;14 (2007) 1476-1486.

37. DENTALI, F. [et al] – Combined aspirin-oral anticoagulant therapy compared with oral anticoagulant therapy alone among patients at risk for cardiovascular disease: a meta-analysis of randomized trials. **Arch Intern Med.** 167;2 (2007) 117-124.

38. COOPER, N.J. [et al] – Mixed comparison of stroke prevention treatments in individuals with nonrheumatic atrial fibrillation. **Arch Intern Med.** 166;12 (2006) 1269-1275.

39. LAFUENTE-LAFUENTE, C. [et al] – Antiarrhytmic drugs for maintaining sinus rhythm after cardioversion of atrial fibrillation: a systematic review of randomized controlled trials. **Arch Intern Med.** 166;7 (2006) 719-728.

40. REID, C.M. [et al] – Oxycodone for cancer-related pain: meta-analysis of randomized controlled trials. **Arch Intern Med.** 166;8 (2006) 837-843.

41. PALMER, S.C. [et al] – Meta-analysis: erythropoiesis-stimulating agents in patients with chronic kidney disease. **Ann Intern Med.** 153;1 (2010) 23-33.

42. MATCHAR, D.B. [et al] – Systematic review: comparative effectiveness of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers for treating essential hypertension. **Ann Intern Med.** 148;1 (2008) 16-29.

43. HART, R.G.; PEARCE, L.A.; AGUILLAR, M.I. – Metaanalysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. **Ann Intern Med.** 146;12 (2007) 857-867.

44. DENTALI, F. [et al] – Metaanalysis: anticoagulant prophylaxis to prevent symptomatic venous thromboembolism in hospitalized medical patients. **Ann Intern Med.** 146;4 (2007) 278-288.

45. CEGLIA, L.; LAU, J.; PITTAS, A.G. – Meta-analysis: efficacy and safety of inhaled insulin therapy in adults with diabetes mellitus. **Ann Intern Med.** 145;9 (2006) 665-675.

46. LIM, W. [et al] – Meta-analysis: low-molecular-weight heparin and bleeding in patients with severe renal insufficiency. **Ann Intern Med.** 144;9 (2006) 673-684.

47. BOLLAND, M.J. [et al] – Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. **BMJ**. 341:c3691 (2010) doi:10.1136/bmj.c3691.

48. JEFFERSON, T. [et al] – Neuraminidase inhibitors for preventing and treating influenza in healthy adults: systematic review and metaanalysis. **BMJ**. 339:b5106 (2009) doi:10.1136/bmj.b5106.

49. STONE, M. [et al] – Risk of suicidality in clinical trials of antidepressants in adults: analysis of proprietary data submitted to US Food and Drug Administration. **BMJ**. 339:b2880 (2009) doi:10.1136/bmj.b2880.

50. STETTLER, C. [et al] – Drug eluting and bare metal stents in people with and without diabetes: collaborative network meta-analysis. **BMJ**. 337:a1331 (2008) doi:10.1136/bmj.a1331.

51. CANONICO, M. [et al] – Hormone replacement therapy and risk of venous thromboembolism in postmenopausal women: systematic review and meta-analysis. **BMJ**. 336;7655 (2008) 1227-1231.

52. STRIPPOLI, G.F.M. [et al] – Effects of statins in patients with chronic kidney disease: meta-analysis and meta-regression of randomised controlled trials. **BMJ**. 336;7645 (2008) 645-651.

53. EURICH, D.T. [et al] – Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: systematic review. **BMJ**. 335;7618 (2007) 497-501.

54. WALTER, M.A. [et al] – Effects of antithyroid drugs on radioiodine treatment: systematic review and meta-analysis of randomised controlled trials. **BMJ**. 334;7592 (2007) 514-520.

55. ARROLL, B.; KENEALY, T. – Are antibiotics effective for acute purulent rhinitis? Systematic review and meta-analysis of placebo controlled randomised trials. **BMJ**. 333;7562 (2006) 279-282.

56. KEARNEY, P.M. [et al] – Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. **BMJ**. 332;7553 (2006) 1302-1308.

57. GLASS, J. [et al] – Sedative hypnotics in older people with insomnia: meta-analysis of risks and benefits. **BMJ**. 331;7526 (2005) 1169.

58. MCDONALD, M.A. [et al] – Angiotensin receptor blockers and risk of myocardial infarction: systematic review. **BMJ**. 331;7521 (2005) 873.

59. LI, S.T.L.; GROSSMAN, D.C.; CUMMINGS, P. – Loperamide: therapy for acute diarrhea in children: systematic review and meta-analysis. **PLoS Med.** 4;3 (2007) e98.

60. KRETSCHMAR, M. [et al] – Frequency of adverse events after vaccination with different vaccinia strains. **PLoS Med.** 3;8 (2006) e272.

CHAPTER III – APIXABAN AND RIVAROXABAN SAFETY AFTER HIP AND KNEE ARTHROPLASTY: A META-ANALYSIS

III. APIXABAN AND RIVAROXABAN SAFETY AFTER HIP AND KNEE ARTHROPLASTY: A META-ANALYSIS

III.1. ABSTRACT

Direct experimental safety comparisons of Xa coagulation factor direct inhibitors, apixaban and rivaroxaban, on their approved therapeutic indications have not been identified. Due to recently raised safety concerns, a meta-analysis was carried out pooling data from studies identified on a MEDLINE and Cochrane Library search in order to better evaluate the safety profile of both drugs. Abstracts from scientific meetings were also searched from 2003 to 2011. Primary and secondary outcome measures were major bleeding and total bleeding, respectively. Relative risks were estimated using random effects models and statistical heterogeneity was estimated with I^2 statistics. Of the 160 screened publications, 12 RCTs were included in which enoxaparin was the active control. For knee arthroplasty, apixaban was associated with significantly fewer major bleeding events (6496 patients, RR 0.56, 95% CI 0.32-0.96) and fewer total bleeding events (6496 patients, RR 0.81, 95% CI 0.67-0.97). There were no significant differences in the incidence of major bleeding events (5699 patients, RR 1.40, 95% CI 0.56-3.52) or in the incidence of total bleeding events for rivaroxaban (5699 patients, RR 1.09, 95% CI 0.91-1.30). No differences were found when thromboprophylaxis after hip replacement was the case. Apixaban seems to be associated with a lower risk of the incidence of hemorrhagic events after total knee arthroplasty. For hip arthroplasty, no differences were found between the studied drugs.

III.2. INTRODUCTION

Patients submitted to major orthopedic surgery, such as elective total knee or hip arthroplasty, represent a group at high risk of venous thromboembolism (VTE) (WARWICK, 2004). Almost half of the patients who underwent arthroplasty are affected by asymptomatic deep venous thrombosis (DVT), although most of these thrombi resolve without long-term complications (GEERTS *et al.*, 2008; HILL and TREASURE, 2010; RASHID *et al.*, 2010). For some patients, propagation of the existing thrombus can cause symptoms as a result of venous occlusion (GINSBERG *et al.*, 2000). Symptomatic VTE is common after discharge from hospital (DOUKETIS *et al.*, 2002; BJORNARA, GUDMUNDSEN and DAHL, 2006). The most frequent cause for readmission to the hospital following total knee arthroplasty is VTE (SEAGROATT *et al.*, 1991).

The provision of thromboprophylaxis during hospitalization has been established as standard of care for the last 2 decades (ANONYMOUS, 1986). With anticoagulant therapy, incidence of fatal pulmonary embolism (PE) decreased to 0.2%, although symptomatic VTE continues to be reported in 1.3% to 10% of patients within 3 months after surgery (GEERTS *et al.*, 2008; SHARROCK et al., 2008). Low molecular weight heparins (LMWHs), fondaparinux, and vitamin K antagonists have been used as pharmacological agents for VTE prophylaxis. Although these therapies have shown to be effective, they are associated with some practical limitations (HIRSH *et al.*, 2008). Low molecular weight heparin and fondaparinux have the inconvenience of subcutaneous administration, which also can increase the risk of injection site hematomas (LASSEN *et al.*, 2010a; JANG and HURSTING, 2005). Furthermore, subcutaneous administration of anticoagulants is difficult to provide after hospital discharge. Vitamin K antagonists are being abandoned in Europe due to concerns about their delayed onset of action, unpredictable pharmacokinetic and pharmacodynamic effects, and need for frequent monitoring (LEVY, KEY and AZRAN, 2010; ANSELL *et al.*, 2008). Mechanical VTE prophylaxis is known to be cumbersome, and its efficacy is found to be lower when compared with anticoagulant therapy, especially after hip arthroplasty (GEERTS *et al.*, 2008).

The specific limitations of the currently available anticoagulant agents led to the development of new therapies for preventing VTE (WEITZ, 2010; BECATTINI, LIGNANI and AGNELLI, 2010; GROSS and WEITZ, 2008). The direct thrombin inhibitor, dabigatran (Pradaxa, Boehringer Ingelheim, Ingelheim am Rheim, Germany), and the oral direct inhibitors of coagulation factor Xa, rivaroxaban (Xarelto, Bayer, Berlin, Germany) and apixaban (Eliquis, Bristol-Myers Squibb/Pfizer EEIG, Uxbridge, UK), were recently approved by the EMA for

thromboprophylaxis after total knee and hip arthroplasty (NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE, 2008; NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE, 2009). Both oral coagulation factorXa direct inhibitors were approved based on evidence provided by phase III RCTs using once daily 40 mg enoxaparin (European regimen) or twice daily 30 mg enoxaparin (North American regimen) as the active control (LASSEN *et al.*, 2009; LASSEN *et al.*, 2010b; ERIKSSON *et al.*, 2008; TURPIE *et al.*, 2009). These studies found that oral coagulation factor Xa direct inhibitors are effective in VTE prevention. However, safety concerns have been raised regarding thromboprophylaxis with rivaroxaban (JENSEN *et al.*, 2011; LOTKE, 2008; CAO *et al.*, 2010; GÓMEZ-OUTES *et al.*, 2009). One study has shown an increased risk of wound complications associated with rivaroxaban (JENSEN *et al.*, 2011). Since the prevalence of fatal PE after total knee arthroplasty is 0.1% and the risk of major and clinically relevant bleeding events with thromboprophylaxis is 3.0%, risk and benefits should be balanced before starting anticoagulation therapies (JOHANSON *et al.*, 2009). The safety of Xa coagulation factor direct inhibitors holding a European market authorization, apixaban and rivaroxaban, have never been evaluated by direct comparisons in RCTs.

The aim of this study was to carry out a meta-analysis in order to comparatively evaluate the safety profile of the direct inhibitors of coagulation factor Xa, apixaban and rivaroxaban, as thromboprophylaxis agents after total knee or hip arthroplasty.

III.3. METHODS

We searched EMA Web site for direct inhibitors of coagulation factor Xa which had been already approved (EUROPEAN MEDICINES AGENCY, 2008; EUROPEAN MEDICINES AGENCY, 2011). Data search and safety analysis were performed according to the therapeutic indication approved by EMA.

III.3.1. SEARCH STRATEGY

MEDLINE and the Cochrane Library were searched from its inception until June 27, 2011 in order to identify relevant studies comparing direct factor Xa inhibitors with enoxaparin. Search terms related with knee and hip arthroplasty (eg 'knee operation', 'knee surgery', 'knee arthroplasty', 'hip arthroplasty', 'hip replacement', and 'hip surgery') were combined with thromboembolism prophylaxis terms, such as 'thrombosis prophylaxis', 'deep venous thrombosis prophylaxis', and 'PE prophylaxis'. Text words, brand names, and manufacturer's coded designations were used to identify both factor Xa inhibitors. All languages were considered in the search strategy. The bibliographic list of all relevant RCTs was hand searched in order to identify additional eligible studies. Study lists from systematic reviews and meta-analysis identified during the search process were also considered. The databases of the American Society of Haematology (starting on the 2004 issue) and the International Society on Thrombosis and Haemostasis (starting on the 2003 issue) were searched in order to identify relevant studies published as abstracts. FDA and EMA publicly available records were searched in order to identify studies that met our inclusion criteria. Articles that were not available were requested to the authors. The electronic databases search strategy is available in the Supplemental Data III.1.

III.3.2. STUDY SELECTION

Literature was searched and relevant studies were examined for further assessment. The studies inclusion criteria were (1) RCTs, (2) patients of all ages undergoing total hip or knee arthroplasty, (3) comparison of safety of factor Xa direct inhibitors with enoxaparin for thromboprophylaxis. Only the oral direct inhibitors of factor Xa holding marketing

authorizations in the EU were evaluated. Both trials with blinded and unblinded design were included. Studies focusing on pharmacokinetic or pharmacodynamic variables were excluded.

III.3.3. QUALITY ASSESSMENT

Quality assessment of studies was based on the recommendations of the Cochrane Handbook of Systematic Review of Interventions on assessing adverse effects (LOKE, PRICE and HERXHEIMER, 2011). The value of trial data on adverse effects relies on 2 major characteristics: the rigor of monitoring for the adverse effects during the study and the completeness of reporting. Allocation concealment and the withdrawal rates were also evaluated.

III.3.4. DATA EXTRACTION

Two reviewers independently extracted data from the included studies. Any disagreements were resolved by consensus or arbitration by a third reviewer. We contacted the authors of studies for missing data when necessary. Data on study characteristics (methodology, included population, study design, and drugs evaluated) and outcomes (bleeding and AEs) during treatment were extracted.

III.3.5. OUTCOME ASSESSED

The primary outcome of this meta-analysis was the incidence of major bleeding beginning after the first dose of the study drug and up to 2 days after the last dose of the study drug (on-treatment period). Major bleeding was defined as bleeding that was fatal, involved a critical organ (eg, retroperitoneal, intracranial, intraocular, and intraspinal), required reoperation or that was clinically overt, extra-surgical site bleeding associated with a fall in hemoglobin level of at least 2 g/dL, calculated from the day 1 postoperative baseline value, or requiring infusion of 2 or more units of whole blood or packed cells. Other safety outcomes included any on-treatment bleeding, any on-treatment clinically relevant nonmajor bleeding, drug-related AEs, drug-related serious AEs, and wound complications. Wound complications outcome is the composite of major and/or nonmajor wound bleedings.

Clinically relevant nonmajor bleeding was defined according to the RCTs that evaluated apixaban and rivaroxaban (LASSEN *et al.*, 2009).

III.3.6. STATISTICAL ANALYSIS

Review Manager (RevMan) version 5.1.2 (Cochrane Collaboration, Oxford, UK) was used to calculate RR and 95% CIs for all the primary and secondary outcomes throughout the meta-analysis. All reported P values are 2-sided with significance set at <.05. Statistical heterogeneity was assessed by calculating a chi-square test and the I^2 measure of inconsistency (HIGGINS *et al.*, 2003). Statistical heterogeneity was considered low when 0%< I^2 < 25%, moderate when 25%< I^2 < 50%, and high when I^2 > 50%. We planned to pool data across studies using the DerSimonian and Laird random effects model (DERSIMONIAN and LAIRD, 1986). The publication bias was assessed by examining the funnel plot (BORENSTEIN *et al.*, 2009). We performed a sensitivity analysis to explore the influence on effect size of blinding of outcome assessment and the methodological quality of included trials. For each Xa coagulation factor direct inhibitor, the results of total hip or knee arthroplasty subgroups were compared with the overall meta-analysis. For the sensitivity analysis, RCTs using twice daily enoxaparin 30 mg (North American regimen) as active control were excluded.

III.4. RESULTS

III.4.1. STUDY SELECTION

Figure III.1 presents the flow of the search strategy criteria. The electronic databases searches returned 165 potentially relevant articles. After review of the titles and abstracts, 142 citations were refused; 16 articles were duplicates; 23 articles were selected for further evaluation. After application of inclusion criteria, 12 studies were eligible for inclusion (LASSEN *et al.*, 2009; LASSEN *et al.*, 2010b; ERIKSSON *et al.*, 2008; TURPIE *et al.*, 2009; LASSEN *et al.*, 2007; TURPIE *et al.*, 2005; ERIKSSON *et al.*, 2006a; ERIKSSON *et al.*, 2006b ERIKSSON *et al.*, 2007; KAKKAR *et al.*, 2008; LASSEN *et al.*, 2008). The review of reference lists scientific society's databases did not find any other relevant studies. No further studies were identified in the FDA and EMA publicly available records.

III.4.2. CHARACTERISTICS OF THE INCLUDED STUDIES

The main characteristics of the studies are presented in Table III.1. Study design, duration of treatment, demographic characteristics of patients, drugs under evaluation, and number of participants are described. A total of 12 RCTs were found, of which 8 evaluated the efficacy and safety of rivaroxaban versus enoxaparin and 4 evaluated the efficacy and safety of apixaban versus enoxaparin. These RCTs included 28 483 patients, in which 15 586 were randomized to receive rivaroxaban or enoxaparin, and 12 897 were randomized to receive apixaban or enoxaparin. All the RCTs were performed in adult patients undergoing total knee or hip arthroplasty. Five RCTs evaluated rivaroxaban for thromboprophylaxis in patients undergoing total hip replacement and 3 RCTs evaluated rivaroxaban for thromboprophylaxis in patients undergoing total knee replacement. Apixaban was evaluated as thromboprophylaxis agent after total knee arthroplasty in 3 RCTs and 1 RCT evaluated apixaban as thromboprophylaxis agent in patients undergoing total hip replacement.

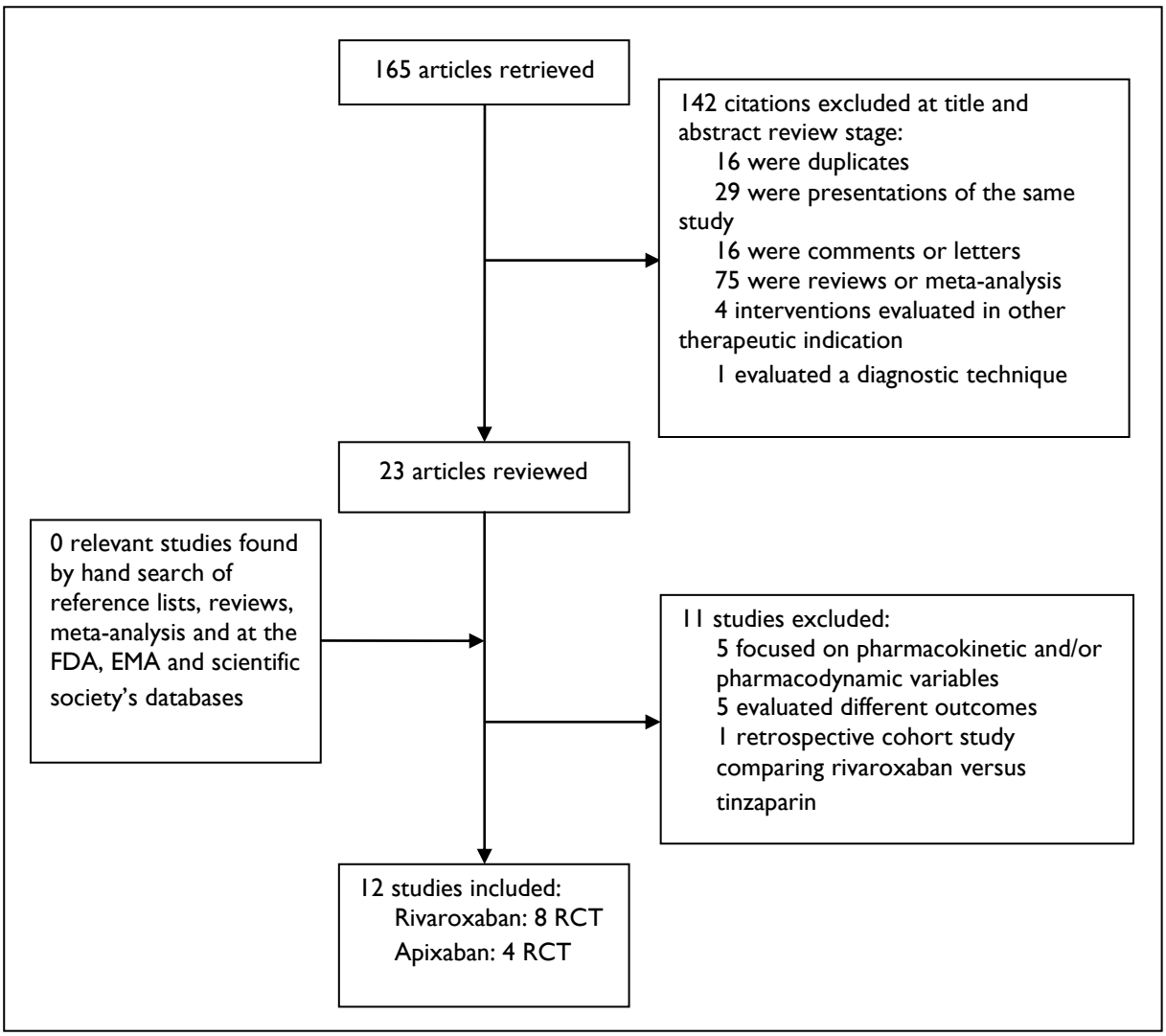


Figure III.1 - Flow diagram of identification of studies for inclusion.

Table III.1 - Characteristics of the studies included in the meta-analysis.

Study	Study design	N° participants	Age, mean	Female, %	Population	Drugs tested	
						Intervention	Enoxaparin
Apixaban							
APROPOS[36]	RCT, double-blind, multicenter	1238	Apix:67.6 Enox:66.5	Apix:68.0 Enox:61.8	Adults, ≥ 18 years old, submitted to total knee arthroplasty.	Apixaban 2.5, 5 or 10 mg, 2 id, or 5, 10 or 20 mg id, p.o., starting 12 to 24h after wound closure, for 10-14 days.	Enoxaparin 30 mg, s.c., every 12h, starting 12 to 24h after wound closure, for 10-14 days.
ADVANCE-1[21]	RCT, double-blind, multicenter	3195	Apix:62.4 Enox:61.8	Apix:62.4 Enox:61.8	Adults, ≥ 18 years old, submitted to total knee arthroplasty.	Apixaban 2.5 mg, 2 id, p.o., starting 12 to 24h after surgery, for 10-14 days.	Enoxaparin 30 mg, s.c., every 12h, starting 12 to 24h after surgery, for 10-14 days.
ADVANCE-2[22]	RCT, double-blind, multicenter	3057	Apix:65.6 Enox:65.9	Apix:71.0 Enox:74.0	Adults, ≥ 18 years old, submitted to total knee arthroplasty.	Apixaban 2.5 mg, 2 id, p.o., starting 12 to 24h after wound closure, for 10-14 days.	Enoxaparin 40 mg, s.c., daily, starting 12h before surgery and restarted according investigators standard of care, for 10-14 days.
ADVANCE-3[12]	RCT, double-blind, multicenter	5407	Apix:60.9 Enox:60.6	Apix:52.8 Enox:53.8	Adults, ≥ 18 years old, submitted to total hip arthroplasty.	Apixaban 2.5 mg, 2 id, p.o., starting 12 to 24h after wound closure, for 31-39 days.	Enoxaparin 40 mg, s.c., daily, starting 12h before surgery and restarted according investigators standard of care, for 31-39 days.
Rivaroxaban							
RECORD1[23]	RCT, double-blind, multicenter	4541	Riva:63.1 Enox:63.3	Riva:55.2 Enox:55.8	Adults, ≥ 18 years old, submitted to total hip arthroplasty.	Rivaroxaban 10 mg, 1 id, p.o., starting 6 to 8h after wound closure, for 31-39 days.	Enoxaparin 40 mg, once daily, starting 12h before surgery, restarting 6 to 8h after wound closure, for 31-39 days.
RECORD2[41]	RCT, double-blind, multicenter	2509	Riva:61.4 Enox:61.6	Riva:54.3 Enox:53.0	Adults, ≥ 18 years old, submitted to total hip arthroplasty.	Rivaroxaban 10 mg, 1 id, p.o., starting 6 to 8h after wound closure, for 31-39 days.	Enoxaparin 40 mg, once daily, starting 12h before surgery, restarting 6 to 8h after wound closure, for 10-14 days.
RECORD3[42]	RCT, double-blind, multicenter	2531	Riva:67.6 Enox:67.6	Riva:70.2 Enox:66.3	Adults, ≥ 18 years old, submitted to total knee arthroplasty.	Rivaroxaban 10 mg, 1 id, p.o., starting 6 to 8h after wound closure, for 10-14 days.	Enoxaparin 40 mg, once daily, starting 12h before surgery, restarting 6 to 8h after wound closure, for 10-14 days.

RECORD4[24]	RCT, double-blind, multicenter	3148	Riva:64.4 Enox:64.7	Riva:66.0 Enox:64.1	Adults, ≥ 18 years old, submitted to total knee arthroplasty.	Rivaroxaban 10 mg, 1 id, p.o., starting 6 to 8h after wound closure, for 10-14 days.	Enoxaparin 30 mg, s.c., every 12h, starting 12 to 24h after wound closure, for 10-14 days.
Eriksson et al. (2007)[40]	RCT, open-label, multicenter	641	Riva:67.0 Enox:64.0	Riva:51.0 Enox:88.0	Men aged ≥ 18 years old and postmenopausal women submitted to elective, primary total hip arthroplasty.	Rivaroxaban 2.5, 5, 10, 20, and 30 mg, every 12 h, p.o., starting 6 to 8h after wound closure, and 30 mg, every 24h, starting 6 – 8h after wound closure, for 5 - 9 days.	Enoxaparin 40 mg, once daily, starting in the evening before surgery, restarting 6 to 8h after wound closure, for 5-9 days.
Eriksson et al. (2) (2006)[39]	RCT, double-blind, multicenter	873	Riva:64.0 Enox:65.6	Riva:63.0 Enox:64.0	Men aged ≥ 18 years old and postmenopausal women submitted to elective, primary total hip arthroplasty.	Rivaroxaban 5, 10, 20, 30 and 40 mg id, p.o., starting 6 to 8h after wound closure, for 5 - 9 days.	Enoxaparin 40 mg, once daily, starting in the evening before surgery, restarting 6 to 8h after wound closure, for 5-9 days.
Eriksson et al. (1) (2006)[38]	RCT, double-blind, multicenter	722	Riva:64.0 Enox:65.0	Riva:54.0 Enox:59.0	Men aged ≥ 18 years old and postmenopausal women submitted to elective, primary total hip arthroplasty.	Rivaroxaban 2.5, 5, 10, 20, and 30 mg, every 12 h, p.o., starting 6 to 8h after wound closure, for 5 - 9 days.	Enoxaparin 40 mg, once daily, starting in the evening before surgery, restarting 6 to 8h after wound closure, for 5-9 days.
Turpie et al. (2005)[37]	RCT, double-blind, multicenter	621	Riva:66.0 Enox:66.0	Riva:64.0 Enox:55.0	Men aged ≥ 18 years old and postmenopausal women submitted to elective, primary total knee arthroplasty.	Rivaroxaban 2.5, 5, 10, 20, and 30 mg, every 12 h, p.o., starting 6 to 8h postsurgery, for 5 -9 days.	Enoxaparin 30 mg, s.c., every 12h, starting on the morning after surgery, for 5-9 days.

Rivaroxaban treatment schedules for thromboprophylaxis were comparable among the included RCTs. The patients of the rivaroxaban group received the first dose 6 to 8 hours after skin wound closure. To avoid clinical heterogeneity, for the dose-ranging studies, only the patients that received a total daily dose of 10 mg were included in the analysis. In our study, all the patients received 10 mg/d of rivaroxaban (single dose or 5 mg twice day).

In the 4 apixaban RCT, the apixaban treatment group received the first dose 12 to 24 hours after wound closure. In the apixaban dose-ranging study, only the patients that received 2.5 mg twice daily were included in the analysis. All the patients included in our study treated with apixaban received 2.5 mg twice a day.

The included treatment arms of apixaban and rivaroxaban correspond to the approved total daily dosage for thromboprophylaxis after total hip or knee arthroplasty, which is 5 mg for apixaban, given 2.5 mg twice daily, and 10 mg for rivaroxaban, given once daily or 5 mg twice daily.

Clinical trials included in the meta-analysis used either the enoxaparin dose and regimen approved for use in Europe (40 mg once daily, first dose received 12 hours or the evening before the surgery and medication resumed 6-8 hours after wound closure) or the regimen approved for use in North America (30 mg twice daily, first dose received on the morning after surgery or 12 or 24 hours after wound closure). The enoxaparin European regimen was administrated in 6 RCTs that evaluated rivaroxaban and in 2 RCTs that evaluated apixaban. The enoxaparin (North American regimen) was administrated in 2 RCTs that evaluated rivaroxaban and in 2 RCTs that evaluated apixaban.

III.4.3. QUALITY ASSESSMENT OF THE INCLUDED STUDIES

The quality assessment of the included trials is presented in Table III.2. All but 1 RCT were double blind. Allocation concealment was adequate in 9 trials. In 3 trials, allocation concealment was unclear. Information on withdrawal rates was available for all trials and ranged from 9.5% to 44.1%. The monitoring of bleeding events was performed in all trials. Besides bleeding events, few adverse effects were specifically monitored. All trials used an independent adjudication committee masked to allocation to assess the outcomes.

III.4.4. PUBLICATION BIAS

We examined the funnel plot (standard error [SE] of log RR plotted against RR) and did not find evidence of publication bias.

III.4.5. SAFETY EVALUATION RESULTS

Results of the meta-analysis comparing apixaban and rivaroxaban with enoxaparin are shown in Table III.3 and Table III.4.

According to the results, apixaban presented a more favorable safety profile compared with enoxaparin in the following outcomes: major bleeding events, all bleeding events, and clinically relevant nonmajor bleeding events for thromboprophylaxis after total knee arthroplasty. No significant differences were found when thromboprophylaxis after total hip arthroplasty was the case. For rivaroxaban, no statistically significant differences in safety profile were found when compared with enoxaparin.

III.4.6. SENSITIVITY ANALYSIS

The sensitivity analysis limited to double-blind RCTs did not change the results. After removing a phase II, dose-ranging RCT, there were no significant differences for the incidence of clinically relevant nonmajor bleeding events between apixaban and enoxaparin thromboprophylaxis after total knee arthroplasty (6193 patients, RR: 0.75, 95% CI: 0.56-1.00, P = 0.05). For all other outcomes in knee or hip arthroplasty thromboprophylaxis, the removing of any other phase II, dose-ranging, study did not change the results.

For the sensitivity analysis, studies that compared direct inhibitor of coagulation factor Xa with twice daily enoxaparin 30 mg were excluded (Supplmental Data III.2). The removal of RCTs using enoxaparin on the North American regimen as active control did not significantly affect any outcome with rivaroxaban after total hip arthroplasty. One RCT compared rivaroxaban and enoxaparin on European regimen for thromboprophylaxis after total knee arthroplasty (LASSEN *et al.*, 2008). The results of that RCT were similar to those obtained in this meta-analysis, for all outcomes. One RCT compared apixaban with enoxaparin European regimen for thromboprophylaxis after total knee arthroplasty (LASSEN *et al.*, 2010). The results of this RCT were different from those obtained in this meta-

analysis. According to the results of that RCT, there were no significant differences in the incidence of bleeding outcomes when apixaban was compared with once daily enoxaparin 40 mg (major bleeding events: 3009 patients, RR: 0.65, 95% CI: 0.28-1.49, P = 0.30; all bleeding events: 3009 patients, RR: 0.83, 95% CI: 0.65-1.06, P = 0.14; clinically relevant nonmajor bleeding events: 3009 patients, RR: 0.76, 95% CI: 0.52-1.12, P = 0.17). After removing the RCTs that used enoxaparin on North American regimen as active control, no statistically significant differences were observed for wound bleeding rates, drug-related AE rates, and drug-related serious AE rates for both apixaban and rivaroxaban.

Table III.2 - Quality assessment of included studies.

Study	Design	Allocation concealment	Adverse event monitoring	Adjudication of adverse events	Withdrawal rate, %
ADVANCE-1[21] Apixaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was bleeding during treatment (major, clinically relev. nonmajor, minor). Other outcomes were elevated liver enzymes, arterial thromboembolic events during treatment and f-up.	Independent central adjudication committee unaware of patient's assigned treatment.	27.6% 29.2%
ADVANCE-2[22] Apixaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was bleeding during treatment (major, clinically relev. nonmajor, minor). Other outcomes were elevated liver enzymes, arterial thromboembolic events during treatment and f-up.	Independent central committee unaware of patient's assigned treatment. Patients screened daily while in hospital.	36.0% 35.0%
ADVANCE-3[12] Apixaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was bleeding during treatment (major, clinically relev. nonmajor, minor). Other outcomes were elevated liver enzymes, arterial thromboembolic events and thrombocytopenia during treatment and f-up.	Independent central committee unaware of patient's assigned treatment. Patients screened daily while in hospital.	28.0% 29.0%
APROPOS[36] Apixaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was major bleeding during treatment. Minor bleeding, any bleeding, potentially significant non-overt bleeding, and AEs during treatment were also assessed.	Independent central committee unaware of patient's assigned treatment. Patients screened daily while in hospital. Safety was assessed via the review of all reported AEs, laboratory test results, and adjudicated bleeding events.	27.4% 28.3%
RECORD1[23] Rivaroxaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was major bleeding during treatment. Other safety outcomes were any bleeding, nonmajor bleeding, hemorrhagic wound complications, AEs and death during treatment. During treatment and f-up were assessed laboratory variables and cardiovascular events.	Independent central adjudication committee unaware of patient's assigned treatment.	29.6% 31.5%
RECORD2[41] Rivaroxaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was major bleeding during treatment. Other safety outcomes were any bleeding, nonmajor bleeding, hemorrhagic wound complications, AEs and death during treatment. During treatment and f-up were assessed laboratory variables and cardiovascular events.	Independent central adjudication committee unaware of patient's assigned treatment. Cardiovascular events were independently and blindly adjudicated.	23.2% 23.5%
RECORD3[42] Rivaroxaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was major bleeding during treatment. Other safety outcomes were any bleeding, nonmajor bleeding, hemorrhagic wound complications, AEs and death during treatment. During treatment and f-up were assessed laboratory variables and cardiovascular events.	Independent central adjudication committee unaware of patient's assigned treatment.	27.6% 27.6%
RECORD4[24] Rivaroxaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was major bleeding during treatment. Secondary outcome was clinically relev. nonmajor bleeding. Other safety outcomes were any bleeding, any nonmajor bleeding, hemorrhagic wound complications, AEs and death during treatment. During treatment and f-up were assessed laboratory variables and cardiovascular events.	Central independent adjudication committees masked to allocation assessed all outcomes.	29.2% 28.9%

Eriksson et al. (2007)[40] Rivaroxaban Enoxaparin	Open-label	Unclear	Primary safety outcome was major, post-operative bleeding during treatment. Secondary outcome was clinically relev. nonmajor bleeding. Other safety outcomes were any bleeding, any nonmajor bleeding, hemorrhagic wound complications, AEs and death during treatment. During treatment and f-up were assessed laboratory variables and cardiovascular events.	Study drug allocation was not revealed to the adjudication committees, who performed their assessments in a blinded manner.	 9.5% 25%
Eriksson et al.(2) (2006)[39] Rivaroxaban Enoxaparin	Double-blind	Unclear	Primary safety outcome was major, post-operative bleeding during treatment. Other safety outcomes were clinically relev. nonmajor bleeding events and minor bleeding events, hematology and clinical chemistry laboratory tests, including liver function and coagulation tests. Post-operative blood loss and transfusion volumes were documented during treatment period.	All bleeding events were assessed centrally by the Bleeding Event Adjudication Committee. All adjudication committees were independent and blinded to treatment allocation.	 20.5% 31.8%
Eriksson et al.(1) (2006)[38] Rivaroxaban Enoxaparin	Double-blind	Unclear	Primary safety outcome was major, postoperative bleeding during treatment. Other safety outcomes were clinically relev. nonmajor bleeding events and minor bleeding events, hematology and clinical chemistry laboratory tests, including liver function and coagulation tests, and serious treatment emergent AEs. Post-operative blood loss and transfusion volumes were documented during treatment period.	All bleeding events were assessed centrally by the Bleeding Event Adjudication Committee. All adjudication committees were independent and blinded to treatment allocation.	 44.1% 32.6%
Turpie et al. (2005)[37] Rivaroxaban Enoxaparin	Double-blind	Adequate	Primary safety outcome was major, postoperative bleeding during treatment. Other safety outcomes were clinically relev. nonmajor bleeding events and minor bleeding events. Post-operative blood loss and transfusion volumes were documented during treatment period.	All bleeding events were assessed centrally by a blinded independent bleeding event committee.	 19.8% 19.7%

Table III.3 - Outcomes of meta-analysis comparing apixaban and enoxaparin.

Outcomes	RCTs n	Apixaban n/N (%)	Enoxaparin n/N (%)	Heterogeneity P	Heterogeneity I^2	RR I.C. 95%	RR P
Major bleeding events							
Knee	3	20/3251 (0.65%)	36/3245 (1.11%)	0.64	0%	0.56 [0.32-0.96]	0.03
Hip	1	22/2673 (0.82%)	18/2659 (0.68%)	-	-	1.22 [0.65-2.26]	0.54
All bleeding events							
Knee	3	195/3251 (5.99%)	242/3245 (7.46%)	0.94	0%	0.81 [0.67-0.97]	0.02
Hip	1	323/2673 (12.08%)	334/2659 (12.56%)	-	-	0.93 [0.81-1.08]	0.34
Clinically relevant nonmajor bleeding events							
Knee	3	79/3251 (2.43%)	107/3245 (3.30%)	0.68	0%	0.74 [0.56-0.99]	0.04
Hip	1	109/2673 (4.08%)	120/2659 (4.51%)	-	-	0.90 [0.70-1.16]	0.43
Drug-related AEs	2	534/3097 (17.24%)	558/3096 (18.02%)	0.81	0%	0.96 [0.86-1.06]	0.40
Drug-related serious AEs	2	27/3097 (0.87%)	41/3096 (1.32%)	0.50	0%	0.66 [0.41-1.07]	0.09
Wound bleedings	3	167/5770 (2.89%)	208/5755 (3.61%)	0.25	27%	0.78 [0.61-1.00]	0.05

Table III.4 - Outcomes of meta-analysis comparing rivaroxaban and enoxaparin.

Outcomes	RCTs n	Rivaroxaban n/N (%)	Enoxaparin n/N (%)	Heterogeneity P	Heterogeneity I^2	RR I.C. 95%	RR P
Major bleeding events							
Knee	3	17/2848 (0.60%)	12/2851 (0.42%)	0.28	22%	1.40 [0.56-3.52]	0.47
Hip	5	13/3795 (0.34%)	8/3904 (0.20%)	0.44	0%	1.70 [0.67-4.32]	0.27
All bleeding events							
Knee	3	229/2848 (8.04%)	210/2851 (7.37%)	0.90	0%	1.09 [0.91-1.30]	0.36
Hip	5	247/3795 (6.51%)	232/3904 (5.94%)	0.38	5%	1.10[0.92-1.33]	0.30
Clinically relevant nonmajor bleeding events							
Knee	3	75/2848 (2.63%)	61/2851 (2.14%)	0.95	0%	1.23 [0.88-1.72]	0.22
Hip	5	117/3795 (3.08%)	95/3904 (2.43%)	0.37	7%	1.20[0.89-1.63]	0.23
Drug-related AEs	4	971/6183 (1.57%)	970/6200 (15.65%)	0.50	0%	1.00 [0.92-1.09]	0.95
Drug-related serious AEs	2	39/2448 (1.59%)	36/2468 (1.46%)	0.21	37%	1.07 [0.60-1.91]	0.82
Wound bleedings	6	105/6399 (1.64%)	106/6494 (1.63%)	0.64	0%	0.99 [0.76-1.29]	0.94

III.5. DISCUSSION

Clinical guidelines recommend pharmacological prophylaxis for patients undergoing total knee and hip arthroplasty for at least 10 days after the surgery (GEERTS *et al.*, 2008; HILL *et al.*, 2010; JOHANSON *et al.*, 2009). New oral anticoagulants, such as apixaban and rivaroxaban, may provide a more suitable antithrombotic therapy and increase patient compliance, when compared with available alternatives (LMWH and vitamin K antagonists).

Several RCTs compared rivaroxaban versus enoxaparin for thromboprophylaxis after total knee or hip arthroplasty, reporting higher efficacy of rivaroxaban when compared with both European and North American enoxaparin regimens (ERIKSSON *et al.*, 2008; TURPIE *et al.*, 2009; KAKKAR *et al.*, 2008; LASSEN *et al.*, 2008). A previous meta-analysis confirmed that rivaroxaban was superior to enoxaparin as a thromboprophylaxis agent after total hip and knee surgery (CAO *et al.*, 2010). However, a higher number of bleeding events associated with rivaroxaban was identified. As a consequence, the authors do not recommend the use of rivaroxaban in patients susceptible to hemorrhage.

Apixaban was proven to be at least as effective as enoxaparin for thromboprophylaxis after total knee or hip arthroplasty (LASSEN *et al.*, 2010a; LASSEN *et al.*, 2009; LASSEN *et al.*, 2010b). Although in ADVANCE 1 study apixaban was not shown to be superior to enoxaparin (North American regimen) after knee arthroplasty, it was associated with a lower bleeding risk (LASSEN *et al.*, 2009). When compared with enoxaparin European regimen, apixaban was superior in preventing thromboembolism after knee and hip arthroplasty without increased bleeding risk (LASSEN *et al.*, 2010a; LASSEN *et al.*, 2010b). Another published meta-analysis points out apixaban as effective as enoxaparin for thromboprophylaxis after total knee arthroplasty (HUANG *et al.*, 2011). In this study, apixaban is associated with significantly fewer major bleeding events. These findings raised the need to comparatively evaluate the safety profiles of apixaban and rivaroxaban, once both the drugs were proven to be efficacious in preventing VTE events.

Our meta-analysis included enoxaparin RCTs, in the absence of studies comparing directly both drugs. The results suggest that thromboprophylaxis with apixaban after total knee arthroplasty is associated with a lower risk of major, clinically relevant nonmajor, and total bleeding events, when compared with rivaroxaban. No differences were observed when apixaban and rivaroxaban were compared for thromboprophylaxis after total hip arthroplasty.

In order to avoid clinical heterogeneity of the studies, 2 subgroup meta-analyses were carried out according to the approved therapeutic indications (hip and knee surgery). A sensitivity analysis was also performed aiming at preventing erroneous interpretations of the results. The removing of phase II, dose-ranging, RCTs from the meta-analysis did not significantly altered the results. Eight RCTs (2 with apixaban and 6 with rivaroxaban) used enoxaparin according to the European regimen (40 mg, once daily) and 4 RCTs (2 with apixaban and 2 with rivaroxaban) used enoxaparin according to the North American regimen (30 mg, twice daily). The indirect comparison of apixaban with rivaroxaban based on the enoxaparin European regimen significantly altered the results. These findings could suggest that twice daily enoxaparin 30 mg is associated with a higher risk of bleeding. Although both the enoxaparin regimens have never been directly compared, this risk was also observed in a previous meta-analysis (HUANG et al., 2011). Nonetheless, indirect comparisons between the 2 inhibitors of coagulation factor Xa based on both enoxaparin regimens (European and North American) consistently present the trend for lower bleeding risk in patients treated with apixaban.

The overall incidence of drug-related adverse reactions of any cause was similar for both drugs. Apixaban was found to be less associated with wound hemorrhages, although this difference did not reach statistical significance. However, when major and nonmajor wound hemorrhages are observed separately, such difference was no longer observed.

Preclinical studies have shown that both rivaroxaban and apixaban are highly selective for factor Xa (BECATTINI, LIGNANI and AGNELLI, 2010). Although it is suggested that rivaroxaban could be associated with an increased factor Xa inhibitory potential (BARRET et al., 2010), this may cause differences in the efficacy and safety profile of both drugs. Therefore, there is a rationale to compare both rivaroxaban and apixaban safety profiles, since differences in the incidence of PE and major bleeding events can change benefit-risk balance that supports therapeutic decisions.

For the phase II dose-ranging studies, both apixaban and rivaroxaban were compared with enoxaparin regimens (LASSEN *et al.*, 2007; TURPIE *et al.*, 2005; ERIKSSON *et al.*, 2006a; ERIKSSON *et al.*, 2006b; ERIKSSON *et al.*, 2007). Based on their phase II programs, rivaroxaban 10 mg once daily and apixaban 2.5 mg twice daily have proven to have similar efficacy and safety to enoxaparin. Therefore, an indirect comparison can be established between rivaroxaban and apixaban safety profile based on their approved daily doses.

The findings of the present study are based on a pooled analysis of 28 483 patients. Safety outcomes considered in this meta-analysis were those defined in the included RCTs.

To our knowledge, this is the first study comparing the safety of the 2 marketed direct inhibitors of coagulation factor Xa. The findings of this meta-analysis may be useful to more accurately establish benefit-risk ratios of both drugs and in the individualization of drug therapy.

The present study has limitations. First, although it includes the results of well-designed RCTs with a large number of patients, some relevant clinical outcomes such as rates of wound infection and wound healing were not assessed in all the included RCTs (JENSEN *et al.*, 2011). Second, only 1 RCT evaluated the safety of apixaban in the thromboprophylaxis after total hip arthroplasty (LASSEN *et al.*, 2010a). Although the RCT included a large number of patients, the availability of more studies would strength the analysis. Third, some heterogeneity between RCTs was found. Such differences could be due to differences in prophylactic treatment duration but also to different enoxaparin regimens (North American and European) used as active control. However, heterogeneity should always be taken into account since each RCT included different populations. Fourth, the number of eligible studies to perform a sensitivity analysis is few. Although an indirect comparison between apixaban and rivaroxaban had been done based on the enoxaparin European regimen, the sample of both included trials could not be powered enough to detect significant differences.

Bleeding is a major concern in patients submitted to thromboprophylaxis after hip or knee arthroplasty after hospital discharge (GEERTS *et al.*, 2008; HILL *et al.*, 2010; JOHANSON *et al.*, 2009). New direct inhibitors of the coagulation factor Xa have proved to be effective in reducing the risk of TVE in a single, unmonitored dose, given orally, which can lead to a more effective anticoagulant therapy. The results of this study suggest that apixaban may be a safer alternative than rivaroxaban for thromboprophylaxis, when total knee arthroplasty is the case. However, more studies are needed, in particular with direct comparisons, in order to better establish the risk profile of these 2 therapeutic agents.

III.6. BIBLIOGRAPHY

ANONYMOUS – Prevention of venous thrombosis and pulmonary embolism. NIH consensus statement. **JAMA**. 256;6 (1986) 744-749.

ANSELL, J. [et al] – American College of Chest Physicians: Pharmacology and management of the vitamin K antagonists: American college of chest physicians evidence-based clinical practice guidelines. 8th ed. **Chest**. 133;6suppl (2008) 160S-198S.

BARRET, Y.C. [et al] – Clinical laboratory measurement of direct factor Xa inhibitors: anti- Xa assay is preferable to prothrombin time assay. **Thromb Haemost.** 104;6 (2010) 1263-1271.

BECATTINI, C.; LIGNANI, A.; AGNELLI, G. – New oral anticoagulants for the prevention of venous thromboembolism. **Drug Des Devel Ther.** 4 (2010) 49-60.

BJORNARA, B.T.; GUDMUNDSEN, T.E.; DAHL, O.E. – Frequency and timing of clinical venous thromboembolism after major joint surgery. **J Bone Joint Surg Br.** 88;3 (2006) 386-391.

BORENSTEIN, M. [et al] – **Introduction to meta-analysis.** Chichester, England: John Wiley & Sons, 2009. ISBN 978-0-470-05724-7.

CAO, Y.B. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after total hip or knee arthroplasty: a meta-analysis of randomized controlled trials. **Eur J Clin Pharmacol.** 66;11 (2010) 1099-1108.

DERSIMONIAN, R.; LAIRD, N. – Meta-analysis in clinical trials. **Control Clin Trials.** 7;3 (1986) 177-188.

DOUKETIS, J.D. [et al] – Short-duration prophylaxis against venous thromboembolism after total hip or knee replacement: a meta-analysis of prospective studies investigating symptomatic outcomes. **Arch Intern Med.** 162;13 (2002) 1465-1471.

ERIKSSON, B.I. [et al] – A once-daily, oral, direct Factor Xa inhibitor, rivaroxaban (BAY 59-7939), for thromboprophylaxis after total hip replacement. **Circulation**. 114;22 (2006b) 2374-2381.

ERIKSSON, B.I. [et al] – Dose-escalation study of rivaroxaban (BAY 59-7939) - an oral, direct Factor Xa inhibitor - for the prevention of venous thromboembolism in patients undergoing total hip replacement. **Thromb Res.** 120;5 (2007) 685-693.

ERIKSSON, B.I. [et al] – Oral, direct Factor Xa inhibition with BAY 59-7939 for prevention of venous thromboembolism after total hip replacement. **J Thromb Haemost.** 4;1 (2006a) 121-128.

ERIKSSON, B.I. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. **N Engl J Med.** 358;26 (2008) 2765-2775.

EUROPEAN MEDICINES AGENCY – Assessment report for Eliquis. Committee for Medicinal Products for Human Use (CHMP), 2011.

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Eliquis. EMEA/543519/2008, Committee for Medicinal Products for Human Use (CHMP), 2008.

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Xarelto. EMEA/543519/2008, Committee for Medicinal Products for Human Use (CHMP), 2008.

GEERTS, W.H. [et al] – Prevention of venous thromboembolism: American college of chest physicians evidence-based clinical practice guidelines. 8th ed. **Chest.** 133;6suppl (2008) 381S-453S.

GINSBERG, J.S. [et al] – Postthrombotic syndrome after hip or knee arthroplasty: a cross-sectional study. **Arch Intern Med.** 160;5 (2000) 669-672.

GÓMEZ-OUTES, A. [et al] – Rivaroxaban versus enoxaparin after total knee arthroplasty. **Lancet**. 374;9691 (2009) 682.

GROSS, P.L.; WEITZ, J.I. – Newanticoagulants for treatment of venous thromboembolism. **Arterioscler Throm Vasc Biol.** 28;3 (2008) 380-386.

HIGGINS, J.P. [et al] – Measuring inconsistency in meta-analysis. **BMJ**. 327;7414 (2003) 557-560.

HILL, J.; TREASURE, T. – Reducing the risk of venous thromboembolism in patients admitted to hospital: summary of NICE guidance. **BMJ**. 340:c95. (2010) doi: 10.1136/bmj.c95.

HIRSH, J. [et al] – American college of chest physicians: parental anticoagulants: American college of chest physicians evidence-based clinical practice guidelines. 8th ed. **Chest.** 133;6suppl (2008) 141S-159S.

HUANG, J. [et al] – Apixaban versus enoxaparin in patients with total knee arthroplasty: a meta-analysis of randomised trials. **Thromb Haemost.** 105;2 (2011) 245-253.

JANG, I.K.; HURSTING, M.J. – When heparins promote thrombosis: review of heparin-induced thrombocytopenia. **Circulation**. 111;20 (2005) 2671-2683.

JENSEN, C.D. [et al] – Return to theatre following total hip and knee replacement, before and after introduction of rivaroxaban: a retrospective cohort study. **J Bone Joint Surg Br.** 93;1 (2011) 91-95.

JOHANSON, N.A. [et al] – Prevention of symptomatic pulmonary embolism in patients undergoing total hip or knee arthroplasty. **J Am Acad Orthop Surg.** 17;3 (2009) 183-196.

KAKKAR, A.K. [et al] – Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial. **Lancet**. 372;9632 (2008) 31-39.

LASSEN, M.R. [et al] – Apixaban or enoxaparin for thromboprophylaxis after knee replacement. **N Engl J Med.** 361;6 (2009) 594-604.

LASSEN, M.R. [et al] – Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. **N Engl J Med.** 363;26 (2010a) 2487-2498.

LASSEN, M.R. [et al] – Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. **Lancet**. 375;9717 (2010b) 807-815.

LASSEN, M.R. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. **N Engl J Med.** 358;26 (2008) 2776-2786.

LASSEN, M.R. [et al] – The efficacy and safety of apixaban, an oral, direct factor Xa inhibitor, as thromboprophylaxis in patients following total knee replacement. **J Thromb Haemost.** 5;12 (2007) 2368-2375.

LEVY, J.H.; KEY, N.S.; AZRAN, M.S. – Novel oral anticoagulants: implications in the perioperative settings. **Anesthesiology**. 113;3 (2010) 726-745.

LOKE, Y.K.; PRICE, D.; HERXHEIMER, A. – Adverse effects. In: HIGGINS, Jullian, GREEN, Sally, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration, 2011. (2011) Available from www.cochrane-handbook.org.

LOTKE, P.A. – Rivaroxaban for thromboprophylaxis. **N Engl J Med.** 359;20 (2008) 2174.

NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE – Dabigatran etexilate for the prevention of venous thromboembolism in patients undergoing elective hip and knee surgery. Technology appraisals, TA157, 2008.

NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE – Rivaroxaban for the prevention of venous thromboembolism after total hip or total replacement in adults. Technology appraisals, TA170, 2009.

RASHID, S.T. [et al] – Venous thromboembolism in UK medical inpatients. **JRSM**. 98;11 (2005) 507-512.

SEAGROATT, V. [et al] – Elective total hip replacement: incidence, emergency readmission rate, and postoperative mortality. **BMJ**. 303;6815 (1991) 1431-1435.

SHARROCK, N.E. [et al] – Potent anticoagulants are associated with a higher all-cause mortality rate after hip and knee arthroplasty. **Clin Orthop Relat Res.** 466;3 (2008) 714-721.

TURPIE, A.G. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. **Lancet**. 373;9676 (2009) 1673-1680.

TURPIE, A.G.G. [et al] – BAY 59-7939: an oral, direct Factor Xa inhibitor for the prevention of venous thromboembolism in patients after total knee replacement. A phase II dose-ranging study. **J Thromb Haemost.** 3;11 (2005) 2479-2486.

WARWICK, D. – New concepts in orthopaedic thromboprophylaxis. **J Bone Joint Surg Br.** 86;6 (2004) 788-792.

WEITZ, J.I. – New oral anticoagulants in development. **Thromb Haemost.** 103;1 (2010) 62-70.

III.7. SUPPLEMENTAL DATA III

III.7.1. SUPPLEMENTAL DATA III.1 - SEARCH STRATEGY

Supplemental Table III.1 - Search strategy performed at MEDLINE and Cochrane Library at June 27, 2011.

Search	PubMed	Results
1	*(thrombosis prophylaxis)* **OR** *(deep venous thrombosis prophylaxis)* **OR** *(thromboembolism prophylaxis)* **OR** *(DVT prophylaxis)* **OR** *(pulmonary embolism prophylaxis)* **OR** *(venous thromboembolism prophylaxis)* **OR** *(prevention of venous thromboembolism)* **OR** *(thromboprophylaxis)* **OR** *(prevention of thromboembolic events)*	32247
2	*(knee operation)* **OR** *(knee surgery)* **OR** *(knee arthroplasty)* **OR** *(arthroplasty of knee)* **OR** *(knee total replacement)* **OR** *(total knee replacement)* **OR** *(total replacement of knee)*	49694
3	*(hip arthroplasty)* **OR** *(arthroplasty of hip)* **OR** *(hip replacement)* **OR** *(hip total replacement)* **OR** *(total hip replacement)* **OR** *(hip surgery)* **OR** *(hip operation)*	49530
4	apixaban **OR** (BMS 562247) **OR** eliquis	158
5	rivaroxaban **OR** (BAY 59 7939) **OR** xarelto	349
6	(((#3 **OR** #2) **AND** #1) **AND** #4)	26
7	(((#3 **OR** #2) **AND** #1) **AND** #5)	93
8	#6 **OR** #7	102
Search	**Cochrane Library**	**Results**
1	*((((knee operation)* **OR** *(knee surgery)* **OR** *(knee arthroplasty)* **OR** *(arthroplasty of knee)* **OR** *(knee total replacement)* **OR** *(total knee replacement)* **OR** *(total replacement of knee))* **OR** *((hip arthroplasty)* **OR** *(arthroplasty of hip)* **OR** *(hip replacement)* **OR** *(hip total replacement)* **OR** *(total hip replacement)* **OR** *(hip surgery)* **OR** *(hip operation)))* **OR** *((thrombosis prophylaxis)* **OR** *(deep venous thrombosis prophylaxis)* **OR** *(thromboembolism prophylaxis)* **OR** *(DVT prophylaxis)* **OR** *(pulmonary embolism prophylaxis)* **OR** *(venous thromboembolism prophylaxis)* **OR** *(prevention of venous thromboembolism)* **OR** *(thromboprophylaxis)* **OR** *(prevention of thromboembolic events)))* **AND** *(rivaroxaban* **OR** *(BAY 59-7939)* **OR** *xarelto)*	Reviews: 10 Clinical trials: 44
2	*((((knee operation)* **OR** *(knee surgery)* **OR** *(knee arthroplasty)* **OR** *(arthroplasty of knee)* **OR** *(knee total replacement)* **OR** *(total knee replacement)* **OR** *(total replacement of knee))* **OR** *((hip arthroplasty)* **OR** *(arthroplasty of hip)* **OR** *(hip replacement)* **OR** *(hip total replacement)* **OR** *(total hip replacement)* **OR** *(hip surgery)* **OR** *(hip operation)))* **OR** *((thrombosis prophylaxis)* **OR** *(deep venous thrombosis prophylaxis)* **OR** *(thromboembolism prophylaxis)* **OR** *(DVT prophylaxis)* **OR** *(pulmonary embolism prophylaxis)* **OR** *(venous thromboembolism prophylaxis)* **OR** *(prevention of venous thromboembolism)* **OR** *(thromboprophylaxis)* **OR** *(prevention of thromboembolic events)))* **AND** *(apixaban* **OR** *(BMS 562247)* **OR** *eliquis)*	Reviews: 8 Clinical trials: 9
3	#1 **OR** #2	Reviews: 10 Clinical trials: 53

III.7.2. SUPPLEMENTAL DATA III.2 - RESULTS OF SENSITIVITY ANALYSIS

Supplemental Table III.2 - Results of the sensitivity analysis comparing apixaban and enoxaparin (40 mg once daily) for thromboprophylaxis after total knee arthroplasty (ADVANCE-2 study results).

	RCTs	**Apixaban**	**Enoxaparin**	**Heterogeneity**		**RR**	
Outcomes	n	n/N (%)	n/N (%)	*P*	I^2	I.C. 95%	*P*
Major bleeding events	1	9/1501 (0.60%)	14/1508 (0.93%)	-	-	0.65 [0.28-1.49]	0.30
All bleeding events	1	104/1501 (6.93%)	126/1508 (8.36%)	-	-	0.83 [0.65-1.06]	0.14
Clinically relevant nonmajor bleeding events	1	44/1501 (2.93%)	58/1508 (3.85%)	-	-	0.76 [0.52-1.12]	0.17

CHAPTER IV – A META-ANALYSIS OF SERIOUS ADVERSE EVENTS REPORTED WITH EXENATIDE AND LIRAGLUTIDE: ACUTE PANCREATITIS AND CANCER

IV. A META-ANALYSIS OF SERIOUS ADVERSE EVENTS REPORTED WITH EXENATIDE AND LIRAGLUTIDE: ACUTE PANCREATITIS AND CANCER

IV.1. ABSTRACT

The association between Glucagon-like peptide-1 (GLP-1) agonists, acute pancreatitis (AP), any cancer and thyroid cancer is discussed. This meta-analysis was aimed at evaluating the risk of those serious AEs associated with GLP-1 agonists in patients with type 2 diabetes. MEDLINE, EMBASE, Cochrane Library and clinicaltrials.gov were searched in order to identify longitudinal studies evaluating exenatide or liraglutide use and reporting data on AP or cancer. ORs were pooled using a random-effects model. I^2 statistics assessed heterogeneity. Twenty-five studies were included. Neither exenatide (OR 0.84 [95% CI 0.58–1.22], I^2 = 30%) nor liraglutide (OR 0.97 [95% CI 0.21–4.39], I^2 = 0%) were associated with an in-creased risk of AP, independent of baseline comparator. The pooled OR for cancer associated with exenatide was 0.86 (95% CI 0.29, 2.60, I^2 = 0%) and for liraglutide was 1.35 (95% CI 0.70, 2.59, I^2 = 0%). Liraglutide was not associated with an increased risk for thyroid cancer (OR 1.54 [95% CI 0.40–6.02], I^2 = 0%). For exenatide, no thyroid malignancies were reported. Current available published evidence is insufficient to support an increased risk of AP or cancer associated with GLP-1 agonists. These rare and long-term AEs deserve properly monitoring in future studies evaluating GLP-1 agonists.

IV.2. INTRODUCTION

Pharmacological treatment of type 2 diabetes mellitus usually requires the sequential addition of antihyperglycemic agents (NATHAN *et al.*, 2009). Both the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) consensus algorithm for the treatment of type 2 diabetes mellitus recommends the initiation of metformin and a lifestyle modification program at the time of diagnosis (NATHAN *et al.*, 2009). Sulphonylureas, thiazolidinediones and insulin can be subsequently added to the therapy (NATHAN *et al.*, 2009).

Glucagon-like peptide-1 agonists are a new class of blood-glucose lowering drugs indicated for the treatment of type 2 diabetes mellitus (DRUCKER *et al.*, 2008; MADSBAD *et al.*, 2004). The first in class, exenatide twice-daily (BID) (Byetta™, Amylin Pharmaceuticals, San Diego, CA, USA/Eli Lilly and Company, Indianapolis, IN, USA), was approved by the FDA and by the EMA in 2005 and 2006, respectively (US FOOD AND DRUG ADMINISTRATION, 2005; EUROPEAN MEDICINES AGENCY, 2006). Lately, a once-weekly (QW) presentation of exenatide (Bydureon™) received a market authorization in Europe (2011) and in the United States (2012) (US FOOD AND DRUG ADMINISTRATION, 2012; EUROPEAN MEDICINES AGENCY, 2011). Liraglutide (Victoza™, Novo Nordisk A/S, Bagsværd, Denmark) was authorized by EMA and FDA in 2009 and 2010, respectively (US FOOD AND DRUG ADMINISTRATION, 2010; EUROPEAN MEDICINES AGENCY, 2009). During the clinical development programmes, the GLP-1 agonists have demonstrated the potential to address fasting and postprandial glucose control with weight loss and low risk of hypoglycaemia (EUROPEAN MEDICINES AGENCY, 2006; EUROPEAN MEDICINES AGENCY, 2009; EUROPEAN MEDICINES AGENCY, 2011). However, this new class of antihyperglycaemic drugs has demanded some attention since potentially, although rare, serious AEs have been associated with their use (DRUCKER *et al.*, 2010).

Post-marketing spontaneous reports of acute pancreatitis among patients treated with exenatide BID have been submitted to FDA's Adverse Event Reporting System (FDA-AERS) since 2005 (US FOOD AND DRUG ADMINISTRATION, 2007). Signal generation analyses of this database identified an increased risk for acute pancreatitis associated with exenatide (ELASHOFF *et al.*, 2011; RASCHI *et al.*, 2013). However, further observational longitudinal studies did not confirm such findings (DORE, SEEGER and CHAN, 2009; GARG, CHEN, and PENDERGRASS, 2010; DORE *et al.*, 2011). The post-marketing case reports led to an update of the exenatide' product labeling, on request of FDA (US FOOD AND DRUG ADMINISTRATION,

2009). Acute pancreatitis was also reported in RCTs with liraglutide (PARKS and ROSENBRAUGH, 2010).

Benign thyroid C-cell adenomas were observed in rodents treated with exenatide BID but no carcinomas were reported (EUROPEAN MEDICINES AGENCY, 2006; DRUCKER *et al.*, 2010). Thyroid tumors occurred in rats administered with exenatide QW in carcinogenicity studies (EUROPEAN MEDICINES AGENCY, 2011). During RCTs, unspecified neoplasms have been reported in patients treated with exenatide BID (EUROPEAN MEDICINES AGENCY, 2006). For liraglutide, C-cell hyperplasia and thyroid cancer were observed in pre-clinical toxicology studies (EUROPEAN MEDICINES AGENCY, 2009; BJERRE KNUDSEN *et al.*, 2010). Several cases of thyroid cancer were also reported during the liraglutide clinical development programme (EUROPEAN MEDICINES AGENCY, 2009; PARKS and ROSENBRAUGH, 2010). When approved by FDA, liraglutide label carries a Black Box warning regarding the risk of thyroid c-cell cancer (US FOOD AND DRUG ADMINISTRATION, 2010).

This study was aimed at evaluating the risk of acute pancreatitis, any cancer or thyroid cancer, associated with GLP-1 agonists, exenatide and liraglutide, by carrying out a meta-analysis based on both experimental and observational published studies.

IV.3. METHODS

IV.3.1. LITERATURE SEARCH

MEDLINE and Cochrane Library were searched from its inception until May 24, 2012 in order to identify relevant studies which evaluated GLP-1 agonists holding a market authorization (US FOOD AND DRUG ADMINISTRATION, 2005; EUROPEAN MEDICINES AGENCY, 2006; US FOOD AND DRUG ADMINISTRATION, 2012; EUROPEAN MEDICINES AGENCY, 2011; US FOOD AND DRUG ADMINISTRATION, 2010; EUROPEAN MEDICINES AGENCY, 2009]. Text words, brand names and manufacturer's coded designations were used to identify the medicines. Only literature published in the English language was considered for inclusion in this analysis. In order to ensure that all studies were identified, a second electronic search in the Medline and EMBASE was performed. Search terms related with pancreatitis and with cancer were combined with the medicines designations priori stated. The search terms were identified by consulting the MedDRA dictionary (MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES, 2011). Bibliographic references list of all relevant studies, meta-analyses and reviews were hand searched in order to identify additional eligible articles. The registration site clinicaltrials.gov was searched in order to identify all studies with available results that evaluated exenatide or liraglutide in type 2 diabetes mellitus. We did not seek to identify safety information of GLP-1 agonists beyond published studies. All the studies reporting zero events in the treatment and/or control group were included. The electronic databases search strategy is available in Supplemental Data IV.I.

IV.3.2. STUDY SELECTION AND QUALITY ASSESSMENT

Literature was searched and relevant studies were selected for further assessment. The studies inclusion criteria were: 1 - published in English language; 2 - RCTs or longitudinal observational studies (case-control or cohort studies); 3 - patients of all ages with type 2 diabetes mellitus; 4 - comparison of GLP-1 agonists with a placebo or active control (oral hypoglycaemic agents or insulin) and 5 - effect estimates on acute pancreatitis or cancer associated with GLP-1 agonists use. Only studies with duration of at least 12 weeks were included.

The quality of the retrieved studies was assessed using the checklist proposed by Downs and Black (DOWNS and BLACK, 1998). Studies' methodological quality was assessed as high, moderate or low when the total score was ≥ 20, from 10 to 19, and < 10, respectively. When more than one reference was found for the same study, methodological quality evaluation was based on the total set of information. Two investigators scored the studies independently. Disagreement was resolved by discussion and consensus with a third investigator.

IV.3.3. DATA EXTRACTION AND OUTCOMES ASSESSED

Data on study design, study duration, characteristics of participants, antihyperglycaemic therapy (dosage and treatment duration) and estimated effect measures or specified outcomes was extracted.

The following outcomes were considered: acute pancreatitis, any cancer and thyroid cancer. For any cancer as an outcome, all the events defined as 'Neoplasms benign, malignant and unspecified (including cysts and polyps)' according to the MedDRA dictionary were considered (MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES, 2011). For thyroid cancer, all terms were considered as those defined in the MedDRA dictionary were taking into consideration (MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES, 2011).

IV.3.4. STATISTICAL ANALYSIS

A meta-analysis was performed by pooling ORs with their 95% confidence intervals CIs, using the DerSimonian and Laird random-effects model and assuming that OR was an unbiased estimate of the RR (DERSIMONIAN and LAIRD, 1986). This model was chosen since the validity of tests of heterogeneity can be limited with a small number of component studies and it is more conservative than a fixed-effect model in the presence of between-studies heterogeneity. When more than one adjusted effect estimate was reported, the most adjusted estimate was used. For studies with more than one intervention-arm, the number of events and the number of exposures were added. The same was applied when studies with multiple controls were the case. Between-studies heterogeneity was assessed by calculating a chi-square test and the I^2 measure of inconsistency (HIGGINS *et al.*, 2003). When no events were reported in one or both groups, a continuity correction of 0.5 was added to each cell.

The publication bias was visually examined by a funnel plot and statistically evaluated by Egger's regression asymmetry test (BORENSTEIN *et al.*, 2009; EGGER *et al.*, 1997).

A sensitivity analysis was conducted to explore the influence of the following variables on the summary estimates: studies' design, studies' methodological quality scores, the nature of the comparators (placebo or active control) and different GLP-1 agonists dose regimens (weekly or daily). All reported P values are 2-sided with significance being set as less than 0.05.

Review Manager (RevMan) version 5.1.6 (Cochrane Collaboration, Oxford, UK) and Comprehensive Meta-analysis Version 2 (Biostat, Englewood, NJ, USA) were used for all statistical analysis.

IV.4. RESULTS

The flowchart of the search strategy criteria is presented in Figure IV.1. The electronic databases searches returned 4373 possible eligible references. After excluding for duplicates and screening the titles and abstracts, 179 bibliographic references were selected and full reports were obtained and evaluated in detail against inclusion criteria. A final sample of 40 references was eligible for inclusion, corresponding to 25 studies. No further studies meeting the inclusion criteria were identified throughout the studies back references lists'. Of the included studies in the analysis, 3 were retrospective cohorts and the remaining were RCTs. Two studies directly compared exenatide and liraglutide (Supplemental IV.2 References 13,14,21).

The main characteristics of the studies and their methodological quality are presented in Table IV.1. More than one article can be referred to one study. For some studies, the information from the public database clinicaltrials.gov complemented that reported in published papers (e.g., length of follow-up). The methodological quality was considered "high" for 15 studies and "moderate" for the other 10 studies.

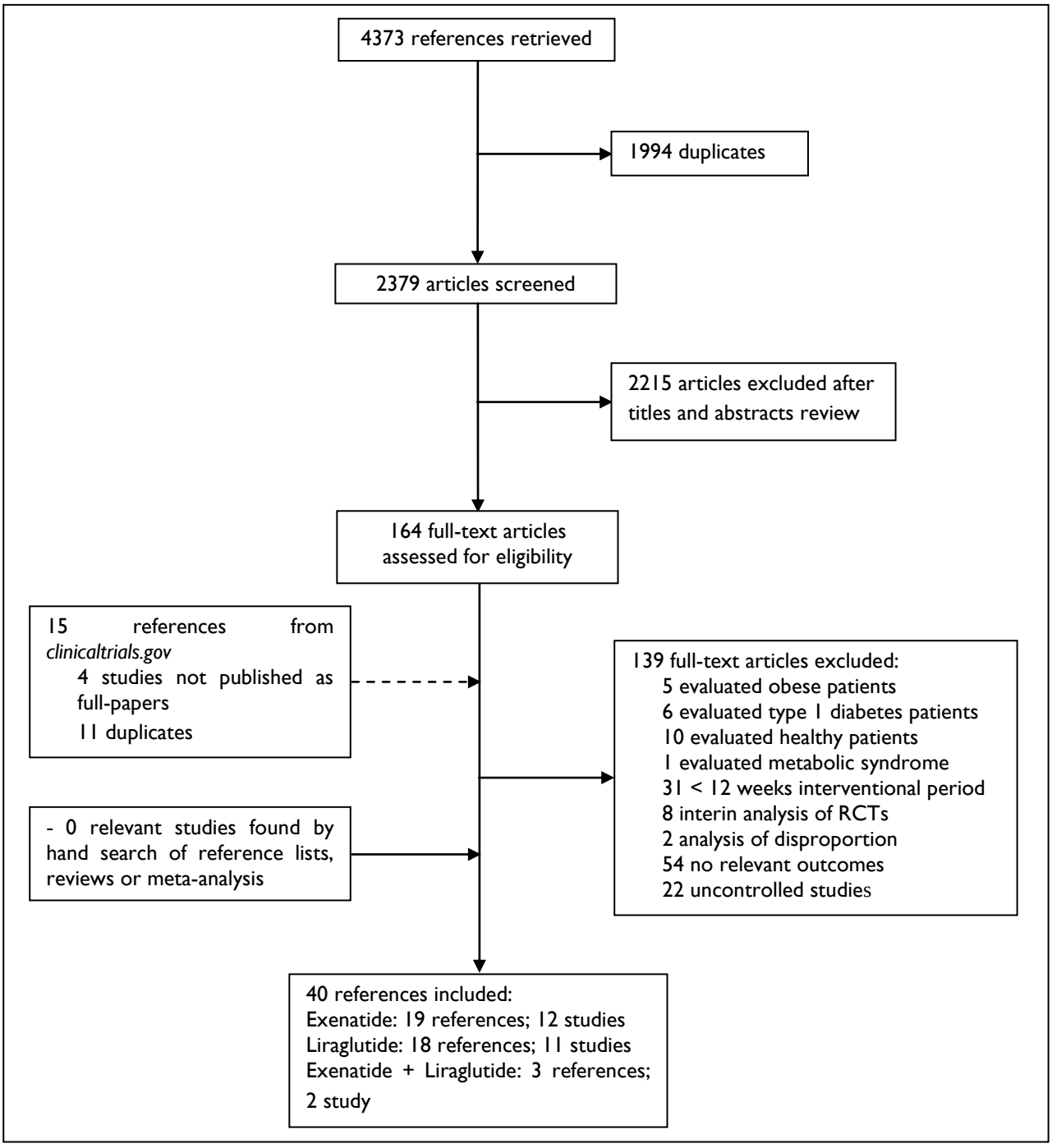


Figure IV.1 - Flow diagram of identification of studies for inclusion.

Table VI.1 - Safety alerts, data sources evaluated, studies included in cumulative meta-analyses and decisions taken by the regulatory authorities.

Date	Regulatory authority	Evidence supporting regulatory decision	Studies included in the cumulative meta-analysis	Label Sections update/regulatory decision
Fluoxetine and cardiovascular birth defects				
25-02-2010	EMA	1 cohort, 1 meta-analysis (9 observational studies)[1,2]	4 case-control, 2 cohort, 1 retrosp. cohort [1,3-8]	Pregnancy section updated
Proton pump inhibitors and bone fractures				
25-05-2010	FDA	5 case-control, 2 cohort, 1 cross-sectional[9-16]	6 case-control, 4 cohort, 1 retrosp. cohort[9-15,17,18,21,22]	Warnings and precautions; dosage recommendations
22-03-2012	EMA	5 case-control, 3 cohort, 2 meta-analysis (11 observational studies), 1 retrosp. cohort[9-15,17-20]		Warnings and precautions
Angiotensin receptor blockers and cancer				
15-07-2010	FDA	3 Meta-analysis of RCTs, 1 cohort[23-26]	23 RCTs, 1 cohort[26-50]	Benefit/risk ratio remains positive
20-10-2011	EMA	1 Meta-analysis of RCTs[23] *RCTs, observational studies (unspecified)* [a]		Benefit/risk ratio remains positive
Pioglitazone and bladder cancer				
17-09-2010	FDA	1 RCT, 1 cohort[51,52]	2 cohort, 1 case-control, 1 RCT[51-54]	Warnings and precautions
16-03-2011	EMA	*Post-marketing spontaneous reports* 2 cohort, 1 RCT, 1 case-control, 1 meta-analysis of RCTs[51-54,b]		Warnings and precautions; contraindications
17-06-2011	Health Canada	*Post-marketing spontaneous reports* 1 RCT, 1 cohort[51,52]		Warnings and precautions; contraindications
18-07-2011	TGA	2 cohort[52,53]		Precautions
Combined hormonal contraceptives containing drospirenone and venous thromboembolism				
27-05-2011	EMA	4 case-control, 2 cohort, 1 retrosp. cohort[55-61]	4 case-control, 2 cohort, 1 retrosp. cohort[55-61]	Warnings and precautions; contraindications
31-05-2011	FDA	3 case-control, 2 cohort, 2 retrosp. cohort[55-60, c]		Warnings and precautions; *remains under revision*
07-06-2011	Health Canada	2 case-control[55,56], *Other studies (unspecified)* [a]		Warnings and precautions; contraindications
06-07-2011	TGA	2 case-control[55,56], *Other studies (unspecified)* [a]		Precautions; contraindications; *remains under revision*
Statins and increased blood sugar				
10-01-2012	EMA	3 RCTs, 1 meta-analysis (13RCTs)[62-65]	16 RCTs, 1 cohort[62,63,67,73-87]	Warnings and precautions
28-02-2012*	FDA	*Review*[66] 3 RCTs, 3 meta-analysis (16 RCTs), 1 cohort, 1 retrosp. cohort[62,67-73]		Warnings and precautions
Proton pump inhibitors and Clostridium difficile associated diarrhea				
08-02-2012	FDA	*Post-marketing spontaneous reports, Case-reports* [a] 17 case-control, 5 retrosp. cohort, 3 cohort, 2 meta-analyses (30 observational studies)[88-113]	24 case-control, 5 retrosp. cohort, 3 cohort[88-100,102-104,106-120]	Warnings and precautions; *remains under revision*
Statins and cognitive side effects				
28-02-2012*	FDA	*Post-marketing spontaneous reports* *2 case report, 1 revision of database with post-marketing spontaneous reports, 1 survey (prospective)*[121-124] 4 RCTs, 2 cohort, 1 case-control, 1 cross-sectional, 1 meta-analysis (7 observational studies), 1 survey (retrospective)[125-134]	6 cohort, 2 case-control[132-139]	Adverse drug reactions section updated
Proton pump inhibitors and pneumonia				
26-07-2012	EMA	*Post-marketing spontaneous reports* *Systematic review*[140]*, randomized clinical trial*[a] 10 case-control, 5 retrosp. cohort, 4 cohort, 3 meta-analyses (12 observational studies), 1 pooled-analysis of RCTs, 1 retrosp., non-comparative study[141-164]	10 case-control, 5 retrosp. cohort, 4 cohort[146-164]	Benefit/risk ratio remains positive; remains under review

Notes: * - issued in the same safety alert; a – reference not provided; b – meta-analysis of RCTs unpublished; c – retrospective cohort unpublished; 24 – published at 29-11-2010; 25 – published at April 2011; w26 – published at 11-04-2011; 52 – published at 21-03-2011; 53 – published at 31-03-2012; 54 – published at 31-05-2012; 89 – study designed as being simultaneously case-control and cohort; 67, 101 – published as short communication; The references list is presented at Supplemental Data VI.1.

	multicenter	had T2 DM	0.9 mg)			Lira 0.6: 60.0 Lira 0.9: 55.5 Placebo: 57.5	Lira 0.6: 37.7 Lira 0.9: 29.5 Placebo: 57.5				
NCT00395746[19]	RCT, double-blind, multicenter	Japanese adults, ≥ 20 years of age, uncontrolled with SU	Liraglutide (0.6, 0.9 mg)	Placebo	52	Lira 0.6: 59.1 Lira 0.9: 61.3 Placebo: 58.6	Lira 0.6: 39.8 Lira 0.9: 32.9 Placebo: 35.2	176/88	1/2	0/0	16
NCT00620282[20]	RCT, double-blind, multicenter	Adults, aged 40-70 years, uncontrolled with METF	Liraglutide (1.8 mg)	Glimepiride/ Placebo	12	Lira 1.8: 57.7 Glime: 57.7 Placebo: 60.3	Lira 1.8: 37.5 Glime: 35.3 Placebo: 37.5	16/33	0/1	0/0	17
NCT01029886[21]	RCT, open-label, multicenter	Adults, ≥ 18 years of age, uncontrolled with METF, SU or both or METF and PIO	Liraglutide (1.8 mg)	Exenatide QW (2 mg)	26	Lira: 56.7 Exe: 56.6	Lira: 45.5 Exe: 44.9	450/461	0/2	0/1	17
Exenatide											
Wenten et al, 2012[22]	Retrospective cohort study	Patients with ≥ 9 months of enrolment without claims for prior pancreatitis and a claim for a new antidiabetic between 03/2005 - 03/2009	Exenatide BID	Other Ads	212	Exe: 52.0 Others Ads: 51.0	Exe: 58.0 Others Ads: 53.0	24237/457797	NR	46/802	16
Dore et al, 2011[23,24] (NCT01077323)	Retrospective cohort study	Patients without claims for prior pancreatic disease and complete medical and pharmacy benefits between 09/2004 - 12/2007	Exenatide BID	Others Ads	123	Exe: UTD Others ADs: UTD	Exe: 55.9 Others ADs: 49.0	25719/234536	NR	11/223	16
Buse et al, 2011[25,26] (NCT00765817)	RCT, double-blind, multicenter	Adults, ≥ 18 years of age, had T2 DM, uncontrolled with IG with or without METF and/or PIO	Exenatide BID	Placebo	30	Exe:59.0 Placebo:59.0	Exe:49.0 Placebo:36.0	137/122	0/0	0/0	24
Gill et al, 2010[27,28] (NCT00516074)	RCT, double-blind, multicenter	Adults, aged 18-75 years, had T2 DM, uncontrolled with METF or a THIAZ or both	Exenatide BID (5 to 10 µg)	Placebo	12	Exe: 57.0 Placebo:54.	Exe: 32.0 Placebo:58.0	28/26	0/0	0/0	20
Garg et al, 2010[29]	Retrospective cohort study	Patients aged 18-63 years, with	Exenatide BID	Others Ads	76	Exe:51.4 Others ADs:	Exe: 56.5 Others ADs:	6545/16244	NR	22/65	15

		pharmacy and medical claims data for a continuous between 01/2007 - 06/2009				52.9	42.8			
Gallwitz et al, 2011[30,31] (NCT00434954)	RCT, open-label, multicenter	Adults, aged 18-90 years, uncontrolled with METF	Exenatide BID (5 to 10 µg)	Premixed insulin aspartat	26	Exe:57.0 PIA:57.0	Exe:UTD PIA:UTD	247/233	2/0	NR	13
DURATION-3[32,33]	RCT, open-label, multicenter	Adults, ≥ 18 years of age, uncontrolled with METF or METF and SU	Exenatide QW (2 mg)	Insuline glargine	84	Exe:58.0 IG:58.0	Exe:48.0 IG:45.0	233/223	1/0	1/0	23
DURATION-2[34]	RCT, double-blind, multicenter	Adults, ≥ 18 years of age, uncontrolled with METF	Exenatide QW (2 mg)	Sitagliptin/ Pioglitazone	26	Exe: 52.0 Sita: 52.0 Pio: 53.0	Exe: 44.0 Sita: 48.0 Pio: 52.0	160/331	0/1	0/2	25
LEAD-6[13,14] (NCT00518882)	RCT, open-label, multicenter	Adults, aged 18-80 years, uncontrolled with METF or SU or both	Exenatide BID (10 µg)	Liraglutide (1.8 mg)	26	Exe: 57.1 Lira: 56.3	Exe: 45.0 Lira: 51.0	232/235	0/3	0/0	23
Kadowaki et al, 2009[35]	RCT, double-blind, multicenter	Japanese adults, aged 20-75 years, had T2 DM, uncontrolled with SU alone or in addition to a BIG or a THIAZ	Exenatide BID (2.5, 5, 10 µg)	Placebo	12	Exe 2.5: 62.2 Exe 5: 60.7 Exe 10: 57.8 Placebo: 60.5	Exe 2.5: 29.7 Exe 5: 32.4 Exe 10: 37.8 Placebo: 25.0	111/40	NR	0/0	21
Bunck et al, 2009[36,37] (NCT00097500)	RCT, open-label, multicenter	Adults, aged 30-75 years, uncontrolled with METF	Exenatide BID (5 to 10 µg)	Insuline glargine	52	Exe: 58.4 IG: 58.3	Exe: 36.1 IG: 33.3	36/33	0/0	1/0	21
Nauck et al, 2007[38]	RCT, open-label, multicenter	Adults, aged 30-75 years, uncontrolled with METF and a SU	Exenatide BID (5 to 10 µg)	Biphasic insulin aspartat	52	Exe: 59.0 BIAsp: 58.0	Exe: 47.0 BIAsp: 51.0	253/248	1/2	NR	20
NCT00577824[39,40]	RCT, double-blind, multicenter	Japanese adults, aged 20-75 years, uncontrolled with SU alone or in addition with a BIG or a THIAZ	Exenatide BID (5, 10 µg)	Placebo	24	Exe 5: 58.5 Exe 10: 59.4 Placebo: 56.3	Exe 5: 31.9 Exe 10: 31.9 Placebo: 31.4	144/35	1/1	0/0	17
NCT01029886[21]	RCT, open-label, multicenter	Adults, ≥ 18 years of age, uncontrolled with	Exenatide QW (2 mg)	Liraglutide (1.8 mg)	26	Exe: 56.6 Lira: 56.7	Exe: 44.9 Lira: 45.5	461/450	2/0	1/0	17

	METF, SU or both or METF and PIO

[a]References for the studies displayed in this table are listed in the Supplement 2. [b]Concealment assignment is referring to the most recent studies´ publication. Studies could be initially double-blinded and then become open-label in the extension phase. [c] Number of patients included in the safety analysis; ID – Intervention drug; C – Control; *n* for "Acute Pancreatitis" and "Cancer" represents the number of patients; M.Q. – Methodological quality assessment; NR – Not reported; The studies LEAD-6 and NCT01029886 compared liraglutide with exenatide. Data from this study was not included in the GLP-1 receptor agonists drug class outcomes estimates; s.c. – subcutaneously; p.o. – orally; Lira – Liraglutide; Sita – Sitagliptin; T2 DM – Type 2 diabetes mellitus; Glime – Glimepiride; SU – Sulphonylurea; Gliben – Glibenclamide; ROSI – Rosiglitazone; Exe – Exenatide; METF – Metformin; GLIMEP - Glimepiride; PIA – Premixed Insulin Aspartat; UTD – unable to determin; IG – Insulin glargine; Sita – Sitagliptin; PIO – Pioglitazone; BIAsp – Biphasic insulin aspartat; BIG; - Biguanide; THIAZ; thiazolidinedione; Ins – Insuline; ADs: antidiabetic drugs

IV.4.1. ACUTE PANCREATITIS

Thirteen studies of exenatide reported acute pancreatitis outcomes (Figure IV.2a). Pooling their estimates yielded an OR of 0.84 (95% CI 0.58–1.22). Similar results were found in the subgroup analysis according to study design for both RCTs (OR 1.70, 95% CI 0.35–8.29) and retrospective cohorts (OR 0.79, 95% CI 0.49–1.27) (Table IV.2). Between-studies heterogeneity accounted for 30% (P = 0.20) of variation in treatment effect, mainly among observational studies (I^2 = 70%, P = 0.03) than between RCTs (I^2 = 0%, P = 0.76). The results did not significantly change from the initial estimates when stratification according to different controls, exenatide dose regimens or when only high methodological quality studies were considered. Non-significant between-studies heterogeneity was observed (Table IV.2).

Twelve liraglutide RCTs reported acute pancreatitis as an outcome (Figure IV.2a). The estimated OR for liraglutide and acute pancreatitis was 0.97 (95% CI 0.21 - 4.39). No significant between-studies heterogeneity was observed. The sensitivity analysis according to different controls and the methodological quality of the studies did not significantly change the results (Table IV.2).

No significant risk reduction was observed in acute pancreatitis for both GLP-1 agonists (OR 0.87, 95% CI 0.64 -1.17).

IV.4.2. ANY CANCER

Ten RCTs studying exenatide reported cancer outcomes (Figure IV.2b). Exenatide was not associated with a significant risk of cancer development (OR 0.86, 95% CI 0.29 - 2.60). The sensitivity analysis according to the different controls, therapeutic regimen and the methodological quality of the studies did not significantly change the results (Table IV.2).

Ten RCTs with liraglutide in type 2 diabetes mellitus reported cancer outcomes (Figure IV.2b). Liraglutide was associated with a statistically non-significant 35% increased risk for any cancer development (OR 1.35, 95% CI 0.70 - 2.59). When liraglutide was compared with different controls, the results did not become statistically significant. However, the stratification of the results becomes statistically significant when only methodological studies of high quality were considered (OR 2.60, 95% CI 1.08 - 6.27) (Table IV.2).

No significant risk reduction was observed in cancer for both GLP-1 agonists (OR 1.24, 95% CI 0.68 - 2.27) and no significant heterogeneity was observed in any of the comparisons (Table IV.2).

IV.4.3. THYROID CANCER

None of the studies evaluating exenatide reported cases of thyroid cancer. Of the studies evaluating liraglutide, five reported cases of thyroid cancer. Nine patients treated with liraglutide were diagnosed with thyroid cancer comparing to one patient who developed this type of cancer and was treated with glimepiride (Supplemental Data IV.2 References 4-6, 9, 10, 13, 14, 16-18). The OR for thyroid cancer occurrence associated with liraglutide treatment was 1.54 (95% CI 0.40-6.02, P = 0.53, I^2 = 0%).

IV.4.4. PUBLICATION BIAS ASSESSMENT

Egger's asymmetry test was not statistically significant for the primary or and most subgroup analyses but was significant for the analysis among exenatide RCTs (P = 0.01) and for once- weekly exenatide regimen studies (P = 0.01) (Table IV.2). Subjective evaluation of publication bias was based on the visual inspection of funnel plot. Few studies were considered for both the analyses, not allowing firm conclusions about the potential publication bias. Regarding cancer risk assessment, large studies are possibly absent for both exenatide and liraglutide.

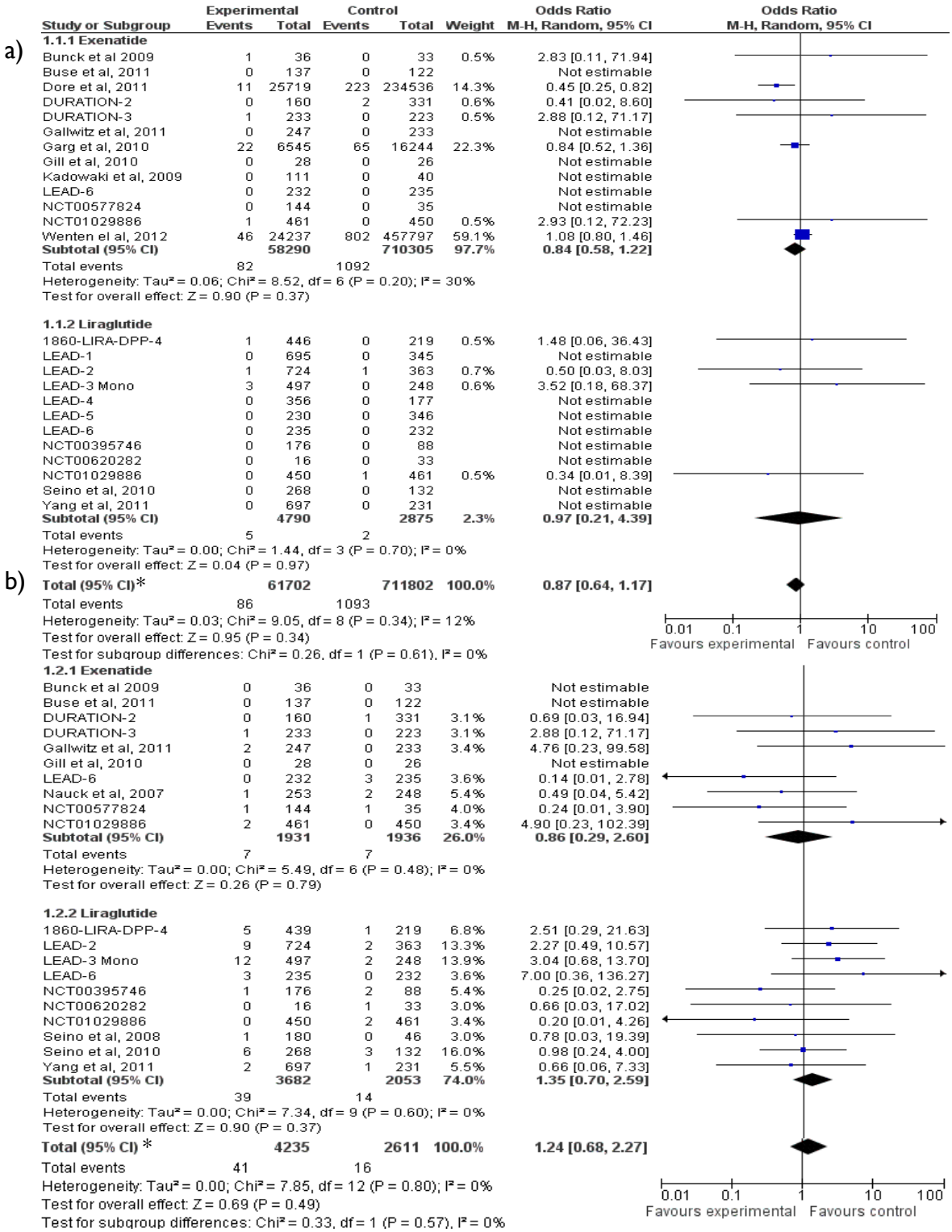


Figure IV.2 - Pooled ORs and 95% CIs of (a) acute pancreatitis and (b) overall cancer associated with GLP-1 agonists.

Note: * For GLP-1 receptor agonists overall pooled results, LEAD-6 and NCT01029886 studies were not included

Table IV.2 - Pooled ORs and 95% CIs of acute pancreatitis and cancer associated with GLP-1 agonists.

GLP-1 receptor agonists	Studies	Odds Ratio (OR)		Heterogeneity		Publication bias*
	N	95% IC	*P*	*P*	I^2	*P*
Acute pancreatitis						
Exenatide						
All studies	13	0.84 [0.58, 1.22]	0.37	0.20	30%	0.94
RCTs	10	1.70 [0.35, 8.29]	0.51	0.76	0%	0.01
Retrospective cohorts	3	0.79 [0.49, 1.27]	0.32	0.03	70%	0.22
vs Insulin	3	2.86 [0.29, 27.86]	0.37	0.99	0%	-
vs OADs	2	0.82 [0.51, 1.33]	0.43	0.65	9%	-
Twice-daily	10	0.81 [0.51, 1.27]	0.36	0.06	59%	0.64
Once weekly	3	1.45 [0.24, 8.90]	0.69	0.60	0%	0.01
High quality	7	1.42 [0.23, 8.81]	0.70	0.61	0%	0.09
Liraglutide						
All studies	12	0.97 [0.21, 4.39]	0.97	0.70	0%	0.97
vs Placebo	6	0.51 [0.02, 12.54]	0.68	-	-	-
vs OADs	3	1.12 [0.20, 6.23]	0.89	0.50	0%	0.58
High quality	3	1.31 [0.24, 7.24]	0.76	0.63	0%	0.63
GLP-1 Agonists	21	0.87 [0.64, 1.17]	0.34	0.34	12%	0.93
Cancer						
Exenatide						
All studies	10	0.86 [0.29, 2.60]	0.79	0.48	0%	0.33
vs Placebo	3	0.24 [0.01, 3.90]	0.31	-	-	-
vs OADs	1	0.69 [0.03, 16.94]	0.82	-	-	-
vs Insulin	4	1.48 [0.29, 7.52]	0.64	0.46	0%	0.22
Twice-daily	7	0.50 [0.12, 2.05]	0.34	0.38	3%	0.78
Once weekly	3	2.20 [0.36, 13.53]	0.40	0.67	0%	0.49
High quality	7	0.56 [0.13, 2.37]	0.43	0.60	0%	0.67
Liraglutide						
All studies	10	1.35 [0.70, 2.59]	0.37	0.60	0%	0.27
vs Placebo	4	0.53 [0.17, 1.65]	0.28	0.86	0%	0.72
vs OADs	6	1.56 [0.74, 3.32]	0.24	0.76	0%	0.82
High quality	5	2.60 [1.08, 6.27]	0.03	0.90	0%	0.84
GLP-1 Agonists	16	1.24 [0.68, 2.27]	0.49	0.80	0%	0.23

Note: * Egger's regression asymmetry test. For GLP-1 agonists pooled results, both LEAD-6 and NCT01029886 studies weren't included.

IV.5. DISCUSSION

The results of this meta-analysis suggest that neither exenatide nor liraglutide increase the risk for acute pancreatitis, when used in the treatment of type 2 diabetes mellitus. However, no conclusions can be drawn since the analysis is based on small studies, possibly underpowered to detect rare AEs.

Our findings are in line with those reported in longitudinal observational studies which evaluated the risk for acute pancreatitis associated with exenatide (DORE, SEEGER and CHAN, 2009; GARG, CHEN, and PENDERGRASS, 2010; DORE *et al.*, 2011). The rates of acute pancreatitis in those studies were less than 0.5%, indicating that this is a rare AE. Our search did not find post-market observational studies for liraglutide.

Although evidence of association has not been established between GLP-1 agonists and acute pancreatitis, a few potentially confounding factors should be considered. Nausea, abdominal discomfort and vomiting are adverse drug reac-tions known to be associated with GLP-1 agonists use (EUROPEAN MEDICINES AGENCY, 2006; EUROPEAN MEDICINES AGENCY, 2009; EUROPEAN MEDICINES AGENCY, 2011). Since these events are also symptoms of acute pancreatitis, its recognition and appropriately diagnose may become difficult (BALANI and GRENDELL, 2008). We only included studies with patients diagnosed with type 2 diabetes mellitus. It was recently documented that having type 2 diabetes puts patients in a higher risk of developing acute pancreatitis, independently of the drug therapy (NOEL *et al.*, 2009). This may raise the question of whether the cases of acute pancreatitis are due to GLP-1 agonists therapy, to type 2 diabetes or to risk factors commonly seen in patients with type 2 diabetes - hypertriglyceridaemia, hyperlipidaemia, obesity, or concomitant medicines (ANDERSON and TRUJILLO, 2010). Considering that GLP-1 agonists were initially approved as type 2 diabetes add-on therapy and the recommendations of clinical guide-lines, patients receiving GLP-1 agonists are more likely to be at more advanced stages of the disease, which increases the risk for pancreatitis, the potential for confounding by indication may be increased, particularly when observational studies are the case (NATHAN *et al.*, 2009; GARG, CHEN, and PENDERGRASS, 2010). Based on spontaneous reports of adverse drug reactions, FDA recommended that the prescribing information of exenatide should include a warning about the risk of acute pancreatitis (US FOOD AND DRUG ADMINISTRATION, 2009). Liraglutide' prescribing information also includes a warning about the risk of pancreatitis, without a specific mention to its onset, type or severity (US FOOD AND DRUG ADMINISTRATION, 2011). This meta-analysis did not find any increased risk for acute pancreatitis associated with both

GLP-1 agonists. Labeling change of exenatide regarding acute pancreatitis required by FDA was supported by spontaneous reports. Therefore, if the increased risk exists, the meta-analysis is unable to identify such risk, since spontaneous reporting data is not considered in the meta-analysis methodology. Similarly the FDA required the market authorization holder of liraglutide to conduct post-approval mechanistic animal studies along with a pharmacoepidemiologic study in order to better assess the risk of acute pancreatitis (PARKS and ROSENBRAUGH, 2010).

Several studies were conducted aiming to explain the mechanisms by which acute pancreatitis could be developed. Butler et al. presented a theoretical model on which GLP-1 agonists could amplify the pancreatic ductal replication already increased by type 2 diabetes mellitus or obesity (BUTLER, DRY and ELASHOFF, 2010; BUTLER *et al.*, 2010). This would increase the risk for low grade chronic pancreatitis that predisposes to acute pancreatitis or pancreatic carcinoma. However, the results of preclinical studies were contradictory, remaining unknown if GLP-1 agonists are associated with a specific pharmacological mechanism that may cause pancreatitis (KOEHLER *et al.*, 2009; NACHNANI *et al.*, 2010; VRANG *et al.*, 2012). In order to avoid misclassification bias, and since the results of pre-clinical studies have shown to be contradictory, only cases reported as acute pancreatitis were included in this meta-analysis.

The possible carcinogenic effect of GLP-1 agonists observed during the pre-clinical studies should be properly evaluated. Moreover, the analysis of disproportion of the FDA-AERS database performed by Elashoff and colleagues (2011) demonstrated an increased risk for thyroid cancer associated with exenatide. This meta-analysis did not identify an increased risk for any cancer associated with exenatide. The risk remained unchanged when the analysis was stratified according to the therapeutic regimens or different comparators. Regarding liraglutide exposure, no difference was observed when data from all studies was integrated or when the results were stratified according to the type of comparator. However, sensitivity analysis restricted to five high methodological quality studies showed an increased risk of cancer from all causes in patients treated with liraglutide. Caution should be taken when interpreting this result, since is the only significant association found, suggesting a possible chance of finding. Several instruments have been developed in order to assess the methodological quality of the studies (JÜNI *et al.*, 1999). The scale of Downs and Black was chosen since it is able to assess both experimental and observational studies (DOWNS and BLACK, 1998).

Although the total number of cancer events was found to be low, a divergence between the risk of cancer associated with exenatide and liraglutide was identified (-14% for exenatide and 35% for liraglutide, both non-significant) (Table 12). Such findings deserve further careful attention. Moreover, when only high quality studies were considered, this difference increases. The present evaluation is based only in data from RCTs since observational studies were not identified in our search strategy. Clinical trials are able to identify the most frequent and common AEs that occurred during the intervention administration. However, considering cancer as a long-latency event, the duration of RCTs and the short period between initial liraglutide exposure and malignancies diagnosis do not allow the establishment of a reliable causality between liraglutide exposure and cancer. No cases of C-cell lesions in thyroid have been documented in patients treated with exenatide. An increased proportion of thyroid carcinomas in patients treated with liraglutide have been reported in the included studies when compared with controls. However, the increased risk was non-statistically significant. As Drucker and colleagues (2010) previously stated, the small number of cases and the lack of biological plausibility raise some doubts between the use of GLP-1 agonists, namely liraglutide, and thyroid cancer occurrence. Moreover, the effects of this drug in humans, particularly in the human thyroid gland, are unknown and difficult to be extrapolated from pre-clinical studies, despite the C-cell hyperplasia in rats (PAROLA, 2009). The findings of this meta-analysis enhance the need for long-term well-designed epidemiological studies devoted to assess the risk for cancer associated with GLP-1 agonists, including thyroid cancer during liraglutide exposure. Additional studies in animals and the establishment of a cancer registry database to monitor the incidence of medullary thyroid cancer associated with liraglutide was required by the FDA (PARKS and ROSENBAUGH, 2010).

This meta-analysis may be subject to several limitations. Of the 22 RCT included, only one included the clinical evaluation of pancreatitis. Despite two RCTs have evaluated the calcitonin levels, none of them were designed to prospectively monitor for malignancies. Pancreatitis and cancer were not defined as an initially outcome measure of RCTs. These events were recorded as serious AEs. The absence of malignancies and/or pancreatitis pre-defined diagnostic criteria can lead to missing events. Moreover, patients enrolled in the RCTs are usually younger and with less comorbidities, being at a lower risk for developing the AEs studied in this meta-analysis when compared with the average patients with type 2 diabetes observed on routine clinical practice. Residual confounding in the included observational studies may extend to the results of this meta-analysis.

Different controls were identified in the RCTs included in this meta-analysis and they might be associated with different risks for acute pancreatitis or cancer, such the case of gliptins or pioglitazone. Because of the heterogeneity of comparators and the relatively small number of acute pancreatitis and cancer events reported in the studies, the stratification of the results at this level is difficult.

Publication bias with regard to acute pancreatitis and cancer is difficult to assess with few studies. In two acute pancreatitis analyses, the results were significant. This may be the case of RCTs unpowered to detect rare events and subsequently creating difficulties in AEs assessments. The European Public Assessment Report (EPAR) of exenatide BID reports that several neoplasms occurred in patients treated with exenatide BID during the clinical development programme, without specifying its type (EUROPEAN MEDICINES AGENCY, 2006). We were unable to find such data in published studies (DEFRONZO *et al.*, 2005; KENDALL *et al.*, 2005; BUSE *et al.*, 2004). This suggests that publication bias may be present in our meta-analysis despites non-significant results observed for this outcome in the Egger's regression asymmetry test. We did not seek to collect data beyond that which is published. However, non-publication of events of such severity turns difficult the correct benefit/risk ratio assessment, and in particular the assessment of the risk for cancer and its subtypes.

Current available published evidence is insufficient to support an increased risk of acute pancreatitis or an increased risk of cancer from all causes associated with GLP-1 agonists. However, there is a growing body of evidence from postmarketing spontaneous reports. Physicians and patients should remain vigilant for episodes of acute pancreatitis or cancer and report any events to the correspondent pharmacovigilance system. Since trials' size, duration and design may not be appropriate to accurately assess the risk of rare or long-term AEs, such acute pancreatitis or cancer, and it is unlikely that randomized trials of GLP-1 agonists designed to detect malignancies will ever exist, clinicians should rely on observational studies in future assessment of the risk of cancer. A rigorous monitoring of these outcomes should be implemented in the future studies since current evidence was not adequately designed to address this issue, precluding any definitive conclusion.

IV.6. BIBLIOGRAPHY

ANDERSON, S.L.; TRUJILLO, M.J. – Association of pancreatitis with glucagon-like peptide-1 agonist use. **Ann Pharmacother.** 44;5 (2010) 904-909.

BALANI, A.R.; GRENDELL, J.H. – Drug-induced pancreatitis: incidence, management, and prevention. **Drug Saf.** 31;10 (2008) 823-837.

BJERRE KNUDSEN, L. [et al] – Glucagon-like peptide-1 receptor agonists active rodent thyroid C-cells causing calcitonin release and C-cell proliferation. **Endocrinology.** 151;4 (2010) 1473-1486.

BORENSTEIN, M. [et al] – **Introduction to meta-analysis.** Chichester, England: John Wiley & Sons, 2009. ISBN 978-0-470-05724-7.

BUSE, J.B. [et al] – Effects of exenatide (Exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. **Diabetes Care.** 27;11 (2004) 2628-2635.

BUTLER, A.E. [et al] – Pancreatic duct replication is increased with obesity and type 2 diabetes in humans. **Diabetologia.** 53;1 (2010) 21-26.

BUTLER, P.C.; DRY, S.; ELASHOFF, R. – GLP-1-based therapy for diabetes: what you do not know can hurt you. **Diabetes Care.** 33;2 (2010) 453-455.

DEFRONZO, R.A. [et al] – Effects of exenatide (Exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. **Diabetes Care.** 28;5 (2005) 1092-1100.

DERSIMONIAN, R.; LAIRD, N. – Meta-analysis in clinical trials. **Control Clin Trials.** 7;3 (1986) 177-188.

DORE, D.D. [et al] – A cohort study of acute pancreatitis in relation to exenatide use. **Diabetes Obes Metab.** 13;6 (2011) 559-66.

DORE, D.D.; SEEGER, J.D.; CHAN, K.A. – Use of a claims-based active drug safety surveillance system to assess the risk of acute pancreatitis with exenatide or sitagliptin compared to metformin or glyburide. **Curr Med Res Opin.** 25;4 (2009) 1019-1027.

DOWNS, S.H.; BLACK, N. – The feasibility of creating a checklist for assessment of the methodological quality of both the randomised and nonrandomised studies of health care interventions. **J Epidemiol Community Health.** 52;6 (1998) 377-384.

DRUCKER, D.J. [et al] – Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. **Lancet.** 372;9645 (2008) 1240-1250.

DRUCKER, D.J. [et al] – Increatin-based therapies for the treatment of type 2 diabetes: evaluation of the risks and benefits. **Diabetes Care.** 33;2 (2010) 428-433.

EGGER, M. [et al] – Bias in meta-analysis detected by a simple, graphical test. **BMJ.** 315 (1997) 629-634.

ELASHOFF, M. [et al] – Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. **Gastroenterology.** 141;1 (2011) 150-156.

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Bydureon. Committee for Medicinal Products for Human Use (CHMP). (2011) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002020/human_med_001457.jsp&mid=WC0b01ac058001d124

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Byetta. Committee for Medicinal Products for Human Use (CHMP). (2006) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000698/human_med_000682.jsp&mid=WC0b01ac058001d124

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Victoza. EMEA/379172/2009. Committee for Medicinal Products for Human Use (CHMP). (2009) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/001026/human_med_001137.jsp&mid=WC0b01ac058001d124

GARG, R.; CHEN, W.; PENDERGRASS, M. – Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. **Diabetes Care.** 33;11 (2010) 2349-2354.

HIGGINS, J.P. [et al] – Measuring inconsistency in meta-analysis. **BMJ.** 327;7414 (2003) 557-560.

JÜNI, P. [et al] – The hazards of scoring the quality of clinical trials for meta-analysis. **JAMA**. 282;11 (1999) 1054-1060.

KENDALL, D.M. [et al] – Effects of exenatide (Exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and sulfonylurea. Diabetes Care 28;5 (2005) 1083-1091.

KOEHLER, J.A. [et al] – Glucagon-like peptide-1 receptor activation modulates pancreatitis-associated gene expression but does not modify the susceptibility to experimental pancreatitis in mice. **Diabetes.** 58;9 (2009) 2148-2161.

MADSBAD, S. [et al] – Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analogue liraglutide (NN221): a 12-week, double-blind, randomized, controlled trial. **Diabetes Care.** 27;6 (2004) 1334-1342.

MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES – Support Documentation: MedDRA v14.0. (2011) Available from http://www.meddra.org.

NACHNANI, J.S. [et al] – Biochemical and histological effects of exendin-4 (exenatide) on the rat pancreas. **Diabetologia.** 53;1 (2010) 153-159.

NATHAN, D.M. [et al] – Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. **Diabetes Care.** 32;1 (2009) 193-203.

NOEL, R.A. [et al] – Increased risk of acute pancreatitis and biliary disease observed in patients with type 2 diabetes. **Diabetes Care.** 32;5 (2009) 834-838.

PARKS, M.; ROSENBRAUGH, C. – Weighting risks and benefits of liraglutide - the FDA's review of a new antidiabetic therapy. **N Engl J Med.** 362;9 (2010) 774-777.

PAROLA, T. – Us Food And Drug Administration . Victoza® (liraglutide injection): human relevance of rodent thyroid C-cell tumors. Centre for Drug Evaluation and Research. (2009) Available from: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM151129.pdf

RASCHI, E. [et al] – The association of pancreatitis with antidiabetic drug use: gaining insight through the FDA pharmacovigilance database. **Acta Diabetol.** 50;4 (2013) 569-577.

US FOOD AND DRUG ADMINISTRATION – Bydureon label and approval history. Centre for Drug Evaluation and Research. (2012) Available from http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails

US FOOD AND DRUG ADMINISTRATION – Byetta (exenatide) injection. Detailed view: safety labelling changes approved by FDA Centre for Drug Evaluation and Research. MedWatch the FDA Safety Information and Adverse Event Reporting Program. (2009) Available from: http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm190571.htm

US FOOD AND DRUG ADMINISTRATION – Byetta drug approval package. Centre for Drug Evaluation and Research. (2005) Available from http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021773_ByettaTOC.cfm

US FOOD AND DRUG ADMINISTRATION – Information for Healthcare Professionals: Exenatide (marketed as Byetta) – 10/2007. Postmarket Drug Safety Information for Patients and Providers. (2007) Available from: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm124712.htm

US FOOD AND DRUG ADMINISTRATION – Victoza (liraglutide [rDNA origin]) Injection: REMS - risk of thyroid C-cell tumors, Acute Pancreatitis. MedWatch the FDA Safety Information and Adverse Event Reporting Program. (2011) Available from: http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm258826.htm.

US FOOD AND DRUG ADMINISTRATION – Victoza drug approval package. Centre for Drug Evaluation and Research. (2010) Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022341s000TOC.cfm

VRANG, N. [et al] – The effects of 13 weeks of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF-rats: no evidence of drug induced pancreatitis. **Am J Physiol Endocrinol Metab.** 303;2 (2012) E253-E264.

IV.7. SUPPLEMENTAL DATA IV

IV.7.1. SUPPLEMENTAL DATA IV.1 - SEARCH STRATEGY

Supplemental Table IV.1 - Search strategy performed at MEDLINE, EMBASE, Cochrane Library and ClinicalTrials.gov at May 24, 2012.

Search	***Medline 1st search strategy***	**Results**
1	(liraglutide) **OR** (victoza) **OR** (NN2211) **OR** (NN 2211) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	**1308**
2	(exenatide) **OR** (byetta) **OR** (bydureon) **OR** (AC2993) **OR** (AC002993) **OR** (AC2993A) **OR** (AC 2993) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	**1883**
Search	***Medline 2nd search strategy***	**Results**
1	(neoplasm) **OR** (neoplasms) **OR** (cancer) **OR** (carcinoma) **OR** (tumour) **OR** (tumours) **OR** (neoplasia) **OR** (neoplasias)	2956013
2	(pancreatitis) **OR** (pancreatitis NOS) **OR** (toxic pancreatitis) **OR** (acute pancreatitis) **OR** (pancreatitis acute)	50663
3	(liraglutide) **OR** (victoza) **OR** (NN2211) **OR** (NN 2211) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	1308
4	(exenatide) **OR** (byetta) **OR** (bydureon) **OR** (AC2993) **OR** (AC002993) **OR** (AC2993A) **OR** (AC 2993) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	1883
5	1 **AND** 3	**79**
6	1 **AND** 4	**115**
7	2 **AND** 3	**31**
8	2 **AND** 4	**49**
Search	***EMBASE***	**Results**
1	(neoplas* **OR** cancer **OR** carcinoma **OR** tumour).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	2527903
2	exp acute pancreatitis/ **OR** pancreatitis/ **OR** toxic pancreatitis.mp. **OR** pancreatitis NOS.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	79073
3	(liraglutide **OR** victoza **OR** NN2211 **OR** NN 2211 **OR** "GLP-1 receptor agonists" **OR** "GLP-1 analogues" **OR** "GLP-1 agonists").mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	2048
4	(exenatide **OR** byetta **OR** bydureon **OR** AC2993 **OR** AC002993 **OR** AC2993A **OR** AC 2993 **OR** "GLP-1 receptor agonists" **OR** "GLP-1 analogues" **OR** "GLP-1 agonists").mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	2320
5	1 **OR** 2	2593671
6	3 **OR** 4	3290
7	5 **AND** 6	**536**
Search	***Cochrane Library***	**Results**
1	(liraglutide) **OR** (victoza) **OR** (NN2211) **OR** (NN 2211) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	**Reviews: 18 Clinical trials: 133**
2	(exenatide) **OR** (byetta) **OR** (bydureon) **OR** (AC2993) **OR** (AC002993) **OR** (AC2993A) **OR** (AC 2993) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	**Reviews: 33 Clinical trials: 192**
Search	***ClinicalTrials.gov***	**Results**
1	(liraglutide) **OR** (victoza) **OR** (NN2211) **OR** (NN 2211) **OR** (GLP-1 receptor	179

	agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	
2	(exenatide) **OR** (byetta) **OR** (bydureon) **OR** (AC2993) **OR** (AC002993) **OR** (AC2993A) **OR** (AC 2993) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists)	238
3	(liraglutide) **OR** (victoza) **OR** (NN2211) **OR** (NN 2211) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists) *Limits*: Studies with results	**14**
4	(exenatide) **OR** (byetta) **OR** (bydureon) **OR** (AC2993) **OR** (AC002993) **OR** (AC2993A) **OR** (AC 2993) **OR** (GLP-1 receptor agonists) **OR** (GLP-1 analogues) **OR** (GLP-1 agonists) *Limits*: Studies with results	**22**

IV.7.2. SUPPLEMENTAL DATA IV.2 - REFERENCES OF THE STUDIES INCLUDED IN THIS META-ANALYSIS

1. PRATLEY, R. [et al] – One year of liraglutide treatment offers sustained and more effective glycaemic control and weight reduction compared with sitagliptin, both in combination with metformin, in patients with type 2 diabetes: a randomised, parallel-group, open-label trial. **Int J Clin Pract.** 65 (2011) 397-407.

2. PRATLEY, R. [et al] – Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. **Lancet.** 375 (2010) 1447-1456.

3. NOVO NORDISK – The effect of liraglutide compared to sitagliptin, both in combination with metformin on glycaemic control in subjects with type 2 diabetes mellitus. (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00700817?term=NCT00700817&rank=1. NLM Identifier: NCT00700817

4. GARBER, A. [et al] – Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. **Lancet.** 373 (2009) 473-481.

5. GARBER, A. [et al] – Liraglutide, a daily human glucagon-like peptide 1 analogue, provides sustained improvements in glycaemic control and weight for 2 years as monotherapy compared with glimepiride in patients with type 2 diabetes. **Diabetes Obes Metab.** 13 (2011) 348-356.

6. NOVO NORDISK – To evaluate the effect of liraglutide versus glimepiride (Amaryl®) on Haemoglobin A1c (LEAD-3). (2012) Available from: http://clinicaltrials.gov/ct2/show/NCT00294723?term=NCT00294723&rank=1. NLM Identifier: NCT00294723

7. YANG, W. [et al] – Liraglutide provides similar glycaemic control as glimepiride (both in combination with metformin) and reduces body weight and systolic blood pressure in Asian population with type 2 diabetes from China, South Korea and India: a 16-week, randomized, double-blind, active control trial. **Diabetes Obes Metab.** 13 (2011) 81-88.

8. NOVO NORDISK – Effect of liraglutide or glimepiride added to metformin on blood glucose control in subjects with type 2 diabetes. (2012) Available from:

http://clinicaltrials.gov/ct2/show/NCT00614120?term=NCT00614120&rank=1. NLM Identifier: NCT00614120

9. SEINO, Y. [et al] – Efficacy and safety of the once-daily human GLP-1 analogue, liraglutide, vs glibenclamide monotherapy in Japanese patients with type 2 diabetes. **Curr Med Res Opin.** 26 (2010) 1013-1122.

10. NOVO NORDISK – Effect of liraglutide on blood glucose control in subjects with type 2 diabetes. (2012) Available from: http://clinicaltrials.gov/ct2/show/NCT00393718?term=NCT00393718&rank=1. NLM Identifier: NCT00393718

11. RUSSEL-JONES, D. [et al] – Liraglutide Effect and Action in Diabetes 5 (LEAD-5) met+SU Study Group. Liraglutide vs insulin glargine and placebo in combination with metformin and sulhonylurea therapy in type 2 diabetes mellitus (LEAD-5 met+SU): a randomised controlled trial. **Diabetologia.** 52 (2009) 2046-2055.

12. ZINMAN, B. [et al] – Efficacy and safety of the human glucagon-like peptide-1 analogue liraglutide in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD-4 Met + TZD). **Diabetes Care.** 32;7 (2009) 1224-1230.

13. BUSE, J.B. [et al] – Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). **Lancet.** 374 (2009) 39-47.

14. NOVO NORDISK – Effect of liraglutide or exenatide added to an ongoing treatment on blood glucose control in subjects with type 2 diabetes (LEAD-6). (2012) Available from: http://clinicaltrials.gov/ct2/show/NCT00518882?term=NCT00518882&rank=1. NLM Identifier: NCT00518882

15. MARRE, M. [et al] – Liraglutide, a once-daily human GLP-1 analogue, added to sulphonylurea over 26 produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with type 2 diabetes (LEAD-1 SU). **Diabet Med.** 26 (2009) 268-278.

16. NAUCK, M. [et al] – Efficacy and safety comparison of liraglutide, glimperide, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (Liraglutide Effect and Action in Diabetes)-2 study. **Diabetes Care.** 32 (2009) 84-90.

17. NOVO NORDISK – To compare the effect of liraglutide when given together with metformin with the effect of metformin alone and with the effect of glimepiride and metformin given together (LEAD-2). (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00318461?term=NCT00318461&rank=1. NLM Identifier: NCT00318461

18. SEINO, Y. [et al] – Dose-dependent improvement in glycemia with once-daily liraglutide without hypoglycemia or weight gain: A double-blind, randomized, controlled trial in Japanese patients with type 2 diabetes. **Diabetes Res Clin Pract.** 81 (2008) 161-168.

19. NOVO NORDISK – Effect of liraglutide in combination with sulphonylurea (SU) on blood glucose control in subjects with type 2 diabetes. (2012) Available from: http://clinicaltrials.gov/ct2/show/NCT00395746?term=NCT00395746&rank=1. NLM Identifier: NCT00395746

20. NOVO NORDISK – The effect of liraglutide on endothelial function in subjects with type 2 diabetes mellitus. (2012) Available from: http://clinicaltrials.gov/ct2/show/NCT00620282?term=NCT00620282&rank=1. NLM Identifier: NCT00620282

21. AMYLIN PHARMACEUTICALS, INC – Safety and Efficacy of Exenatide Once Weekly Versus Liraglutide in Subjects With Type 2 Diabetes. (2013) Available at: http://clinicaltrials.gov/ct2/show/NCT01029886?term=NCT01029886&rank=1. NLM Identifier: NCT01029886

22. WENTEN, M. [et al] – Relative risk of acute pancreatitis in initiators of exenatide twice daily compared with other anti-diabetic medication: a follow-up study. **Diabetic Med.** 29;11 (2012) 1412-1418.

23. DORE, D.D. [et al] – A cohort study of acute pancreatitis in relation to exenatide use. **Diabetes Obes Metab.** 13 (2011) 559-566.

24. AMYLIN PHARMACEUTICALS, INC. – A retrospective cohort study of acute pancreatitis in relation to use of exenatide and other antidiabetic agents. (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT01077323?term=NCT01077323&rank=1. NLM Identifier: NCT01077323

25. BUSE, J.B. [et al] – Use of exenatide in basal insulin-treated patients with type 2 diabetes: a randomized controlled trial. **Ann Intern Med.** 154 (2011) 103-112.

26. AMYLIN PHARMACEUTICALS, INC. – Addition Of Exenatide To Insulin Glargine In Type 2 Diabetes Mellitus. (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00765817?term=NCT00765817&rank=1. NLM Identifier: NCT00765817

27. GILL, A. [et al] – Effect of exenatide on heart rate and blood pressure in subjects with type 2 diabetes mellitus: a double-blind, placebo-controlled, randomized pilot study. **Cardiovasc Diabetol.** 9 (2010) 6.

28. AMYLIN PHARMACEUTICALS, INC. – Addition A Study to Assess the Effect of Exenatide Treatment on Mean 24-Hour Heart Rate in Patients With Type 2 Diabetes. (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00516074?term=NCT00516074&rank=1. NLM Identifier: NCT00516074

29. GARG, R.; CHEN, W.; PENDERGRASS, M. – Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. **Diabetes Care.** 33 (2010) 2349-2354.

30. GALLWITZ, B. [et al] – Exenatide twice daily versus premixed insulin aspart 70/30 in metformin-treated patients with type 2 diabetes: a randomized 26-week study on glycemic control and hypoglycemia. **Diabetes Care.** 34 (2011) 604-606.

31. AMYLIN PHARMACEUTICALS, INC – Effect of exenatide plus metformin vs. insulin aspart plus merformin on glycemic control and hypoglycemia in patients with type 2 diabetes. (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00434954?term=NCT00434954&rank=1. NLM Identifier: NCT00434954

32. DIAMANT, M. [et al] – Once weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes (DURATION-3): an open-label randomised trial. **Lancet.** 375 (2010) 2234-2243.

33. DIAMANT, M. [et al] – Safety and efficacy of once-weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes over 84 weeks. **Diabetes Care.** 35;4 (2012) 683-689.

34. BERGENSTAL, R.M. [et al] – Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial. **Lancet.** 376 (2010) 431-439.

35. KADOWAKI, T. [et al] – Exenatide exhibits dose-dependent effects on glycemic control over 12 weeks in Japanese patients with suboptimally controlled type 2 diabetes. **Endocr J.** 56;3 (2009) 415-424.

36. BUNCK, M.C. [et al] – One-year treatment with exenatide improves β-cell function, compared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial. **Diabetes Care.** 32 (2009) 762-768.

37. AMYLIN PHARMACEUTICALS, INC – Effects of exenatide and insulin glargine in subjects with type 2 diabetes. (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00097500?term=NCT00097500&rank=1. NLM Identifier: NCT00097500

38. NAUCK, M.A. [et al] – A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study. **Diabetologia.** 50 (2007) 259-267.

39. KADOWAKI, T. [et al] – Improved glycemic control and reduced bodyweight with exenatide: A double-blind, randomized, phase 3 study in Japanese patients with suboptimally controlled type 2 diabetes over 24 weeks. **J Diabetes Invest.** 2;3 (2011) 210-217.

40. AMYLIN PHARMACEUTICALS, INC – Efficacy and safety of exenatide in Japanese patients with type 2 diabetes who are treated with oral antidiabetic(s). (2013) Available from: http://clinicaltrials.gov/ct2/show/NCT00577824?term=NCT00577824&rank=1. NLM Identifier: NCT00577824

CHAPTER V – SOURCES OF INFORMATION USED BY REGULATORY AGENCIES ON THE GENERATION OF DRUG SAFETY ALERTS

V. SOURCES OF INFORMATION USED BY REGULATORY AGENCIES ON THE GENERATION OF DRUG SAFETY ALERTS

V.1. ABSTRACT

The study of the grounds on which data regulatory authorities base their decisions on drug safety evaluations is an important clinical and public health issue. The aim of this study was to review the type and publication status of data sources supporting benefit/risk ratio reevaluations conducted by the major regulatory authorities on safety issues. A website search was carried out to identify all safety alerts published by the FDA, Health Canada, EMA and the Australian Therapeutics Goods Administration (TGA). Safety alerts were included if the causal relation between a suspected drug exposure and the occurrence of an AE was evaluated for the first time between 2010 and 2012. Type of data sources evaluated by these regulatory authorities, publication status of the data sources and status of the drug label section with respect to updating were evaluated. A total of 59 safety alerts were included in this study. Of these, 33 (56%) were supported by postmarketing spontaneous reports, 24 (41%) evaluated RCTs, 16 evaluated cohort studies (27%), 13 were case-control studies (22%) and 11 evaluated case report/case-series (17%). Twenty-three safety alerts (39%) were issued based. on unpublished evidence, corresponding mainly to postmarketing spontaneous reports. The "Warnings and precautions section" was the drug label section most frequently updated (n=40; 68%). Despite the different lengths of time taken by the different regulatory authorities to come to similar decisions on the same issues - an issue which would seem to deserve further harmonization - post-marketing spontaneous reports have supported most of the benefit/risk ratio reevaluations, thereby confirming the value of such method in detecting unknown AEs.

V.2. INTRODUCTION

Assessment of the benefit/risk relation is conducted throughout the entire life cycle of a drug, starting from its clinical development and continuing during the post-licensing phase (US FOOD AND DRUG ADMINISTRATION, 2012). Before a market authorization is granted, drugs are studied for a defined therapeutic indication in RCTs with a limited duration and with strict inclusion/exclusion criteria for a relatively small number of patients (MADRE *et al.*, 2006). These are accepted limitations to RCTs, one of which is that not all harmful effects can be easily anticipated (WYSOWSKI and SWARTZ, 2005). Some AEs can only be detected after marketing authorization, and these may be sufficiently serious to require a change in the established benefit/risk relation profile of a particular drug, leading to its label change or even market removal (MOORE, SINGH and FURBERG, 2012).

Post-marketing spontaneous reporting systems are useful to identify rare and/or serious AEs which could not be anticipated during the pre-approval stage (Wysowski and SWARTZ, 2005; AHMAD, MARKS and GOETSCH, 2006). Spontaneous reporting of AEs covers all drugs during their entire life cycle, both the whole population and special subgroups (EDWARDS *et al.*, 2006). However, regulatory authorities recognize that this surveillance system may have limitations, such as underreporting or lack of data on the number of exposed individuals (US FOOD AND DRUG ADMINISTRATION, 2008).

Additional studies are usually needed to confirm safety signals identified through spontaneous reports (EDWARDS *et al.*, 2006; US FOOD AND DRUG ADMINISTRATION, 2008). Indeed, observational studies may better reflect the nature of AEs occurring in clinical practice since they include populations usually underrepresented in RCTs, such as the elderly, pregnant women or patients with comorbidities (MADRE *et al.*, 2006; ROTHWELL et al., 2005; PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006).

Safety signals represent findings and results from "reported information on a possible causal relationship between an AE and a drug, being the relationship unknown or incompletely documented at that time" (EDWARDS and BIRIELL, 1994). A safety signal can also be generated from other sources than post-marketing spontaneous reports, such as pre-clinical data, observational longitudinal studies or even from information on other drugs of the same pharmacological class (BULL, 2007). Therefore, postmarketing data collection and risk assessment are critical steps in characterizing a drug's safety profile and lead to better decisions on which regulatory actions should be implemented (BULL, 2007; US FOOD AND DRUG ADMINISTRATION, 2011). As a consequence, the study of the grounds on which

supporting data have been reviewed by regulatory authorities on clinical safety evaluations is an important public health issue.

The aim of this study was to review the type and publication status of data sources supporting benefit/risk ratio reevaluations conducted by four major regulatory authorities on safety issues evaluated between January 2010 and December 2012.

V.3. METHODS

The websites of four health regulatory authorities and reference data sources were reviewed to identify safety alerts. Data were extracted from the following publicly accessible addresses: the FDA "Drug Safety Communications", "Advisories, warnings and recalls" of Health Canada, EMA "News, press release and public statement archive", monthly reports of the "CHMP' Pharmacovigilance Working Party" and "Pharmacovigilance Risk Assessment Committee", and the TGA "All alerts" and "All recalls" (US FOOD AND DRUG ADMINISTRATION , 2014; HEALTH CANADA, 2013; EUROPEAN MEDICINES AGENCY, 2014; EUROPEAN MEDICINES AGENCY, 2013; THERAPEUTIC GOODS ADMINISTRATION, 2014; THERAPEUTIC GOODS ADMINISTRATION, 2014). Safety alerts were included if the causal relation between a suspected drug exposure and the occurrence of an AE was evaluated for the first time between January 2010 and December 2012. Natural and healthcare products, medical devices, contrast agents, drug-drug interactions, drug-food interactions, medication errors, evaluations of lack of efficacy and AEs occurring during off-label use were not considered for inclusion.

Only safety alerts on drugs with market authorization and simultaneously included in one of the 30 most prescribed drug classes worldwide used in the ambulatory setting were considered for inclusion (Supplemental Data V.1). Drug classes were considered as the second level therapeutic subgroup of the ATC classification system (WHO COLLABORATION CENTRE FOR DRUG STATISTICS METHODOLOGY, 2014). Data on sales of drug classes were requested from IMS (Intercontinental Marketing Services) Health. The bibliographic lists of all relevant safety alerts were hand searched in order to identify additional eligible safety alerts. The website search and the safety alerts selection were conducted by one researcher (Carlos Alves). A second researcher scanned the included safety alerts (Ana Filipa Macedo). Discrepancies were resolved by majority (two out of three) decision involving a third investigator (Francisco Batel Marques).

The following information from each safety alert was extracted: (1) date of first publication; (2) regulatory authority issuing the safety alert; (3) suspected drug(s); (4) AE of interest; (5) type of data source supporting the evaluation, namely: (I) study design; (ii) results for the outcome of interest; (iii) publication status; (6) drug label' section(s) updated. "Drug remains under revision" or "benefit/risk ratio unchanged" were considered when any label change was performed.

Regarding the publication status of the data sources, postmarketing spontaneous reports and studies consulted by regulatory authorities which had not been published at the time of the safety alert disclosure were considered to be unpublished data. Updates of the same safety alert were revised in order to retrieve further information on the regulatory authority decision and/or other data sources evaluated. Two safety alerts were considered to be evaluating the same clinical question when they assessed the same AE for the same suspected drug(s). Each safety alert could have been supported by more than one type of data source. More than one section of the drug label could have been updated. Regulatory authorities could have decided to keep the suspected drug(s) under revision despite labeling changes having been carried out.

V.4. RESULTS

The results of our search of the regulatory authorities' websites are displayed in Figure V.1. A total of 1,204 publications were initially identified, of which 953 were excluded after further review of the titles, subjects and publication dates. This resulted in 251 safety alerts identified as "possibly eligible" for inclusion. After confirming the drug class as one of the 30 most consumed worldwide in ambulatory care, 59 safety alerts were included in the study, of which five were published by TGA, 13 by Health Canada, 16 by FDA and 25 by EMA.

Table V.1 describes the characteristics of the eligible safety alerts. Forty-two different clinical questions were evaluated in the 59 safety alerts, of which 28 clinical questions were evaluated by only one regulatory authority and the remaining 14 by at least two regulatory authorities. Thirty-two different drugs or drug classes were evaluated.

V.4.1. SCIENTIFIC INFORMATION SOURCES EVALUATED IN SAFETY ALERTS

Table V.2 presents the different scientific information sources evaluated by the regulatory authorities. Thirty-three (56 %) safety alerts issued by authorities supported their regulatory decisions on post-marketing spontaneous reports, of which 18 (20%) were based exclusively on this data source. Twenty four (41%) safety alerts evaluated RCTs, eight (14%) of them exclusively. Cohort studies supported 16 (27 %) regulatory decisions, followed by case-control studies (n=13; 22 %) and case report/case-series (n=11; 17 %). Meta-analyses of RCTs, meta-analyses of observational studies, retrospective cohorts and surveys supported regulatory decisions on six (11 %) safety alerts each. Health Canada and EMA did not provide the scientific evidence supporting two evaluations.

The design of studies supporting the review of three safety alerts (5 %) by authorities was not specified.

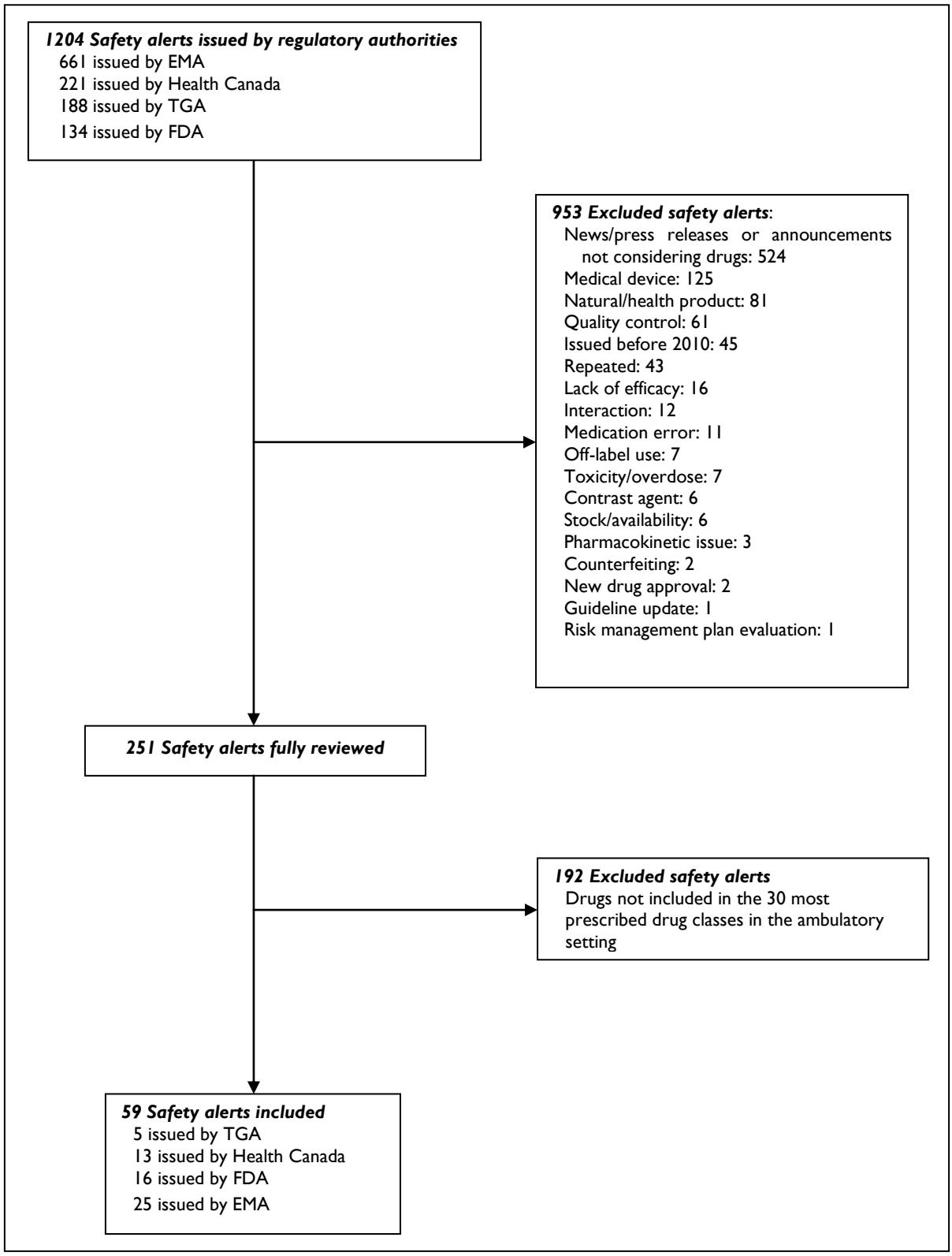


Figure V.1 - Flow diagram of identification of safety alerts for inclusion.

Table V.1 - Safety alerts, data sources evaluated and decisions taken by the regulatory authorities.

Date	Regulatory authority	Risk	Scientific evidence	Label Sections update/regulatory decision
Fluoxetine and cardiovascular birth defects				
25-02-2010	EMA	R > 1	1 cohort, 1 meta-analysis of observational studies[1,2]	Pregnancy section updated
Lamotrigine and increased risk of fatal liver failure				
25-03-2010	EMA	-	Post-marketing spontaneous reports	Benefit/risk ratio remains positive; *remains under revision*
		-	Case reports[a]	
Proton pump inhibitors and bone fractures				
25-05-2010	FDA	R > 1	4 case-control, 2 cohort[3-8]	Warnings and precautions; dosage recommendations
		R ≈ 1	1 case-control, 1 cross-sectional[9,10]	
22-03-2012	EMA	R > 1	4 case-control, 3 cohort, 2 meta-analysis of observational studies, 1 retrospective cohort[3-8,11-14]	Warnings and precautions
		R ≈ 1	1 case-control[9]	
Rosuvastatin and diabetes mellitus				
27-05-2010	EMA	R > 1	1 RCT[15]	Warnings and precautions
Angiotensin receptor blockers and cancer				
15-07-2010	FDA	R > 1	1 Meta-analysis of RCTs[16]	Benefit/risk ratio remains positive
		R ≈ 1	2 Meta-analyses of RCTs, 1 cohort[17-19]	
20-10-2011	EMA	R > 1	1 Meta-analysis of RCTs[16]	Benefit/risk ratio remains positive
		-	*RCTs, observational studies (unspecified)* [a]	
Ketoprofen-containing medicines used topically and photoallergic reactions				
22-07-2010	EMA	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications
Daptomycin and eosinophilic pneumonia				
29-07-2010	FDA	-	Post-marketing spontaneous reports	Warnings and precautions
		-	Case reports[20-23]	
Methylnaltrexone and gastrointestinal perforation				
03-08-2010	Health Canada	-	Post-marketing spontaneous reports	Warnings and precautions
Lamotrigine and aseptic meningitis				
12-08-2010	FDA	-	Post-marketing spontaneous reports	Warnings and precautions
Pioglitazone and bladder cancer				
17-09-2010	FDA	R > 1	1 RCT, 1 cohort[24,25]	Warnings and precautions
16-03-2011	EMA	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications
		R > 1	2 cohort, 1 RCT, 1 case-control, 1 meta-analysis of RCTs[24-27,b]	
17-06-2011	Health Canada	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications
		R > 1	1 RCT, 1 cohort[24,25]	
18-07-2011	TGA	R > 1	2 cohort[25,26]	Precautions
Tinzaparin and increased risk of mortality in elderly patients with renal impairment				
19-10-2010	Health Canada	R > 1	1 RCT[28]	Warnings and precautions
Corticosteroids (inhalational or intranasal use) and psychiatric, behavioral and other systemic adverse reactions				
25-11-2010	EMA	-	Post-marketing spontaneous reports	Warnings and precautions
		-	3 revision of database with post-marketing spontaneous reports ,1 prospective, non-comparative study, 1 review, 1 survey (prospective)[29-34]	

Antipsycothic drugs and abnormal muscle movements and withdrawal symptoms in newborns				
22-02-2011	FDA	-	Post-marketing spontaneous reports	Pregnancy section updated
15-06-2011	Health Canada	-	Post-marketing spontaneous reports	Warnings and precautions
28-07-2011	EMA	-	Post-marketing spontaneous reports	Warnings and precautions; pregnancy section updated
Proton pump inhibitors and hypomagnesemia				
02-03-2011	FDA	-	Post-marketing spontaneous reports	Warnings and precautions
		-	4 case reports, 3 case series, 1 prospective non-comparative study[35-42]	
10-01-2012	EMA	-	Post-marketing spontaneous reports 3 case reports[36,37,43]	Warnings and precautions
Topiramate and congenital malformations (oral clefts)				
02-03-2011	FDA	R > 1	2 Pregnancy registers database study[c]	Warnings and precautions
24-11-2011	EMA	R > 1	2 Pregnancy registers database study[c]	Pregnancy section updated
Combined hormonal contraceptives containing drospirenone and venous thromboembolism				
27-05-2011	EMA	R > 1	3 case-control, 1 cohort[44-47]	Warnings and precautions; contraindications.
		R ≈ 1	1 cohort, 1 retrospective cohort, 1 case-control[48-50]	
31-05-2011	FDA	R > 1	3 case-control, 1 cohort, 1 retrospective cohort[44-47,d]	Warnings and precautions; *remains under revision*
		R ≈ 1	1 cohort, 1 retrospective cohort[48,49]	
07-06-2011	Health Canada	R > 1	2 case-control[44,45]	Warnings and precautions; contraindications; *remains under revision*
		-	*Other studies (unspecified)* [a]	
06-07-2011	TGA	R > 1	2 case-control[44,45]	Precautions; contraindications; *remains under revision*
		-	*Other studies (unspecified)* [a]	
Valproate and impaired cognitive development (in children from mothers who took the medication during pregnancy)				
30-06-2011	FDA	R > 1	2 retrospective cohort, 1 cohort, 1 survey (retrospective)[51-54]	Warnings and precautions
Metoclopramide and tardive dyskinesia				
20-07-2011	Health Canada	-	Post-marketing spontaneous reports	Boxed warning
Citalopram and arrhythmia (QT interval prolongation)				
24-08-2011	FDA	-	Post-marketing spontaneous reports	Warnings and precautions; dosage recommendations
		R > 1	1 RCT[c]	
13-10-2011	Health Canada	R > 1	1 RCT[c]	Warnings and precautions; contraindications; dosage recommendations
27-10-2011	EMA	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications; dosage recommendations
		R > 1	1 RCT[c]	
04-11-2011	TGA	R > 1	1 RCT[c]	Precautions; contraindications; dosage recommendations
Asenapine and hypersensitivity reactions				
01-09-2011	FDA	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications
Atomoxetine and increase in blood pressure and increase in heart rate				
24-10-2011	Health Canada	R > 1	1 RCT[a]	Warnings and precautions; contraindications
02-11-2011	TGA	R > 1	1 RCT[a]	Precautions; contraindications
24-11-2011	EMA	R > 1	1 RCT[a]	Warnings and precautions; contraindications
Antiepileptics and bone disorders (decreased bone mineral density, osteopenia and osteoporosis)				
27-10-2011	EMA	-	Post-marketing spontaneous reports	Benefit/risk ration remains positive
		R > 1	8 retrospective, non-comparative study, 7 case-control, 6 retrospective cohort, 5	

			cohort, 4 prospective, non-comparative study, 2 cross-sectional, 2 survey (retrospective), 1 meta-analysis of observational studies[55-93]	
		R ≈ 1	2 case-control[94,95]	
Fluoroquinolones and muscle weakness (in patients with myasthenia gravis)				
07-11-2011	Health Canada	-	Post-marketing spontaneous reports	Warnings and precautions
Escitalopram and arrhythmia(QT interval prolongation)				
24-11-2011	EMA	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications; dosage recommendations
		R > 1	1 RCT[c]	
07-05-2012	Health Canada	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications
		R > 1	1 RCT[c]	
Rosuvastatin and gynaecomastia				
24-11-2011	EMA	-	Post-marketing spontaneous reports	Adverse events section updated
		-	2 case reports[a]	
		R > 1	4 RCTs[a]	
		R ≈ 1	1 RCT[a]	
Tibolone and venous thromboembolism				
24-11-2011[#]	EMA	-	Post-marketing spontaneous reports	Warnings and precautions; contraindications
		-	RCT[a]	
		R > 1	1 case-control[96]	
Tibolone and myocardial infarction				
24-11-2011[#]	EMA	R > 1	1 cohort, 1 case-control[97]	Warnings and precautions; contraindications
Tibolone and breast cancer and ovarian cancer				
24-11-2011[#]	EMA	R > 1	1 cohort[98]	Warnings and precautions; contraindications
Zolpidem and complex sleep behaviours				
05-12-2011	Health Canada	-	Post-marketing spontaneous reports	Warnings and precautions
Dabigatran and serious bleeding				
07-12-2011	FDA	-	Post-marketing spontaneous reports	*Remains under revision; additional studies required*
		R ≈ 1	1 RCT[99]	
21-03-2012	Health Canada[b]	-	Post-marketing spontaneous reports	Dosage recommendations
24-05-2012	EMA[c]	-	Post-marketing spontaneous reports	Benefit/risk ratio remains positive
Statins and increased blood sugar				
10-01-2012	EMA	R > 1	2 RCTs, 1 meta-analysis of RCTs[15,100,101]	Warnings and precautions
		R < 1	1 RCT[102]	
28-02-2012[*]	FDA	-	Review[103]	Warnings and precautions
		R > 1	3 RCT, 3 meta-analysis of RCTs, 1 cohort, 1 retrospective cohort[101,104-110]	
Proton pump inhibitors and Clostridium difficile associated diarrhea				
08-02-2012	FDA	-	Post-marketing spontaneous reports	Warnings and precautions; *remains under revision*
		-	Case-reports [a]	
		R > 1	14 case-control, 4 retrospective cohort, 2 cohort, 2 meta-analyses of observational studies[111-131]	
		R ≈ 1	3 case-control, 1 cohort, 1 retrospective cohort[132-136]	
Statins and cognitive side effects				
28-02-2012[*]	FDA	-	Post-marketing spontaneous reports	*Adverse drug reactions section updated*
		-	2 case report, 1 revision of database with post-marketing spontaneous reports, 1	

			survey (prospective)[137-140]	
		R > 1	2 RCTs[141,142]	
		R ≈ 1	2 RCTs, 1 meta-analysis of observational studies, 1 survey (retrospective)[143-146]	
		R < 1	2 cohort, 1 case-control, 1 cross-sectional[147-150]	
Lamotrigine and increased risk of sudden unexpected death in patients with epilepsy				
31-05-2012	EMA	-	2 review, 1 case report[151-153]	Benefit/risk ratio remains positive; *remains under revision*
		R > 1	1 case-control[154]	
		R ≈ 1	1 pooled-analysis of observational studies[155,156]	
		R < 1	1 meta-analysis of RCTs[157]	
Donepezil and neuroleptic malignant syndrome				
26-07-2012	EMA	-	Post-marketing spontaneous reports	Warnings and precautions
		-	4 case report[158-161]	
		-	*RCTs, pre-clinical studies*[a]	
Proton pump inhibitors and pneumonia				
26-07-2012	EMA	-	Post-marketing spontaneous reports	Benefit/risk ratio remains positive; remains under review
		-	Systematic review[162], *RCT*[a]	
		R > 1	7 case-control, 3 cohort, 3 meta-analyses of observational studies, 2 retrospective cohort, 1 retrospective, non-comparative study[163-178]	
		R ≈ 1	3 case-control, 3 retrospective cohort, 1 cohort, 1 pooled-analysis of RCTs[179-186]	
Prazepam and glaucoma				
26-07-2012	EMA	-	Post-marketing spontaneous reports	Benefit/risk ratio remains positive
		-	5 case reports, 1 RCT, 1 systematic review[187-193]	
Codeine and life-threatening AEs (in children submitted to tonsillectomy and/or adenoidectomy and who are "ultra-rapid metabolizers")				
15-08-2012	FDA	-	Post-marketing spontaneous reports 2 case report, 1 survey (retrospective)[194-196]	Boxed warning, warnings and precautions, contraindications; pediatric section and patient counseling information
Nicotinic acid/ laropiprant and serious AEs such bleeding (intracranial and gastro-intestinal), myopathy, infections and new-onset diabetes				
21-12-2012	EMA	R > 1	1 RCT[197]	Suspended
			Supporting data sources not provided	
Proton pump inhibitors and Clostridium difficile associated diarrhea				
16-02-2012	Health Canada	-	*Supporting data do not provided*	Warnings and precautions; *remains under revision*
Codeine and life-threatening AEs (in children submitted to tonsillectomy and/or adenoidectomy and who are "ultra-rapid metabolizers")				
05-10-2012	EMA		*Supporting data do not provided*	Remains under revision
			Based upon other regulatory authority evaluation	
Statins and cognitive side effects				
02-03-2012¥	TGA	-	*Based on US FDA safety warning*	*Benefit/risk ratio remains positive*
Statins and increased blood sugar				
02-03-2012¥	TGA	-	*Based on US FDA safety warning*	*Precautions*

Notes: # - issued in the same safety alert; * - issued in the same safety alert; ¥ - evaluated in the same safety warning; OTC – over the counter; a – reference not provided; b – meta-analysis of RCTs unpublished; c - unpublished; d – retrospective cohort unpublished; 17 – published at 29-11-2010; 18 – published at April 2011; 19 – published at 11-04-2011; 25 – published at 21-03-2011; 26, 27 – published at 31-05-2012; 28 - published at 07-04-2011; 97 – study designed as being simultaneously case-control and cohort; 105, 125 – published as short communication; The references list is presented at Supplemental Data V.2.

V.4.2. PUBLICATION STATUS OF DATA SOURCES

The publication status of data sources evaluated by regulatory authorities is presented in Table V.2. Twenty-two (37 %) safety alerts were supported by published and unpublished evidence. Most of the unpublished evidence evaluated by the authorities consisted of postmarketing spontaneous reports (17/22). At the date of their release, the regulatory decisions on four safety alerts were supported by studies (cohort, case-control and meta-analysis of RCTs) subsequently published as full papers.

Twenty-one (36 %) safety alerts were issued based solely on unpublished scientific evidence. The majority of these alerts (16/21) were based on postmarketing spontaneous reports, followed by the results of RCTs (7/21). The regulatory decisions on citalopram and escitalopram and the risk for arrhythmia (QT interval prolongation) were based on postmarketing spontaneous reports and unpublished data from RCTs. Clinical trials supporting regulatory decisions on tinzaparin had not been published when the respective safety alerts were issued.

Fourteen safety alerts were supported exclusively on scientific evidence already published. Observational studies were revised by regulatory authorities in the majority of these alerts (8/14). A safety alert on Atomoxetine was issued by three regulatory authorities which relayed their decision on RCTs sponsored by the drug' market owner but did not provide references.

V.4.3. REGULATORY ACTIONS

Table V.3 describes the different safety regulatory actions and the frequency by which they were applied. The most commonly updated drug label section was the Warnings/Precautions section (n=40; 68 %), followed by the update of the Contraindications section (n=17; 29 %). Updates of the Dosage section due to new recommendations were made in eight evaluations (14 %). New boxed warnings were issued on two occasions (3%). The marketing authorization of an association of drugs was preventively suspended. In eight safety alerts (14 %) the authorities announced that the benefit/risk ratio of the drug remained positive. The safety profile of nine (14 %) drugs/drug classes remains under revision.

The Australian TGA took a regulatory decision upon the evaluation conducted by US FDA on statins and the risk for increased blood sugar and cognitive side effects.

Table V.2 - Data sources and its publication status.

	n = 59			
Type of data sources reviewed			*Exclusive*	
Reported at least one data source	57	97%	-	-
Post-marketing spontenous reports	33	56%	*12*	*20%*
RCT	24	41%	*8*	*14%*
Cohort	16	27%	-	-
Case-control	13	22%	-	-
Case report/case series	11	17%	-	-
Meta-analysis of RCTs	6	10%	-	-
Meta-analysis of observational studies	6	10%	-	-
Retrospective cohort	6	10%	-	-
Survey (prospective or retrospective)	6	10%	-	-
Systematic review	5	9%	-	-
Prospective, non-comparative studies	3	5%	-	-
Cross-sectional	3	5%	-	-
Pregnancy registers database	2	3%	*2*	3%
Retrospective, non-comparative studies	2	3%	-	-
Revision of post-marketing spontaneous reports database	2	3%	-	-
Pre-clinical studies	1	2%	-	-
Unpublished/references not provided	18	31%	-	-
Unspecified design	3	5%	-	-
Based on other regulatory authority warning	1	2%	-	-
Type of data sources not clarified	2	3%	-	-
Publication status of data sources on safety alerts	*n=57**			
Published and unpublished	22	*37%*	-	-
Unpublished	21	*36%*	-	-
Published	14	24%	-	-

* - For the analysis of the publication status of data sources on safety alerts, only those which reported the type of data sources were considered (n=57). Each safety alert could have been supported by more than one type of data source.

Table V.3 - Drug label sections updated by regulatory authorities

	n=59	
Regulatory actions		
Warnings/precautions	40	68%
Contraindications	17	29%
Dosage recommendations	8	14%
Pregnancy section updated	4	7%
Adverse events section updated	2	3%
Boxed warning	2	3%
Patient counseling information	1	2%
Pediatric section	1	2%
Suspension	1	2%
Remains under revision	9	15%
Benefit/Risk ratio remains positive	8	14%
Additional studies required	1	2%

More than one section of the drug label could have been updated. Regulatory authorities could have decided to remain the suspected drug(s) under revision despite labeling changes have been carried out.

V.5. DISCUSSION

The results of this study provide evidence that in the cases of the safety alerts assessed herein regulatory authorities reviewed, either isolated or in combination, several sources of information to support their decisions on safety issues associated with the most widely consumed drug classes in ambulatory care, including published and non-published data. Such sources of information mainly comprised post-marketing spontaneous reports and experimental and observational clinical studies.

Spontaneously notified reports of cases were present in the majority of the benefit/risk ratio reassessments, with a considerable proportion (20%) of safety evaluations conducted exclusively on this source of evidence. This seems to be the case for rare and previously unsuspected situations (WYSOWSKI and SWARTZ, 2005). Such events are prone to be reported by healthcare professionals or patients when they occur within a relatively short period of time after the initiation of the treatment or following a dose increment (MADRE *et al.*, 2006).

The results of this study are similar to those found in other studies, thereby confirming the value of the pharmacovigilance spontaneous reporting systems in providing evidence on iatrogenic risk (MOORE, SINGH and FURBERG, 2012; LESTER *et al.*, 2013). One previous study demonstrated that the results from this surveillance system have provided evidence of serious safety problems, leading not only label changes but also to the withdrawal of drugs from the market (WYSOWSKI and SWARTZ, 2005). Reports of cases may be the only available evidence suggesting an association between a suspected drug and an AE, since no further studies may have been conducted or, if conducted, their results may preclude any definitive conclusions (US FOOD AND DRUG ADMINISTRATION, 2009; DORE, SEEGER and CHAN, 2009; GARG, CHEN, and PENDERGRASS, 2010; DORE *et al.*, 2011). In such cases, regulatory authorities may require risk minimization programmes to prevent more patients from being exposed to unnecessary risk (US FOOD AND DRUG ADMINISTRATION, 2009). However, voluntary reports of suspected adverse drug reactions have limitations. The quality of the data reported may be low, and some events may be more frequently reported than others, such as those which are rare and serious. Additionally, a drug may be subject to an increased number of reports in the early years after being granted a market authorization (AHMAD, MARKS and GOETSCH, 2006). Such limitations lead post-marketing spontaneous

reports to be considered as generating hypotheses rather than confirming them, and additional analytic studies may be required to better assess the safety profile of a drug.

In this study, evidence from RCTs supported a significant number of regulatory decisions. When RCTs constituted the only source of evidence, it was not uncommon that the AE was an end-point of interest of the study (e.g. QT interval prolongation, bleeding, mortality). Clinical trials are considered to be the most reliable source of scientific evidence that can support healthcare policies and clinical practice (GUYATT et al., 2008). However, the majority of RCTs are designed to evaluate the most common AEs occurring early on the treatment.

Observational studies are more likely to be involved in the detection of long-term latency AEs. Moreover, this type of data may better represent the frequency of harmful effects experienced in actual clinical practice (PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006; VANDENBROUCKE and PSATY, 2008). Several regulatory decisions on AEs occurring with a long-latency time from the initiation of the treatment, such as fractures, cardiovascular events or malignancies, were found to be based on observational designs, the majority being cohort and case-control studies. Despite a considerable proportion of the safety alerts being supported by RCTs (41%), this study found that observational data made a relevant contribution towards supporting safety issues, not only in postmarketing reports of cases (56%) but also in longitudinal studies [cohort (27 %) case-control (22 %)] and case reports/case-series (17 %). Since only the most consumed classes of medicines were included in this work, the probability of these drugs being the subject of observational studies is high. Moreover, it is expected that the contribution of observational data to label updates due to safety issues may increase due to the adoption of electronic health records (LESTER *et al.*, 2013).

The quality of the evidence supporting regulatory decisions on drug safety has been discussed (MOORE, SINGH and FURBERG, 2012; WOLFE, 2012; DAL PAN and TEMPLE, 2012). Methodological limitations of the studies may impair causality assessment; this was the conclusion of the EMA on the association between antiepileptic drugs and bone disorders. Most of the regulatory decisions presented in this work were based on the evaluation of evidence which was not immediately published in the scientific literature or which is difficult to access by the scientific community, such as the post-marketing spontaneous reports or studies requested from market authorization holders. As such, the regulatory authority is in an unmatched position to conduct critical analyses. However, access to unpublished data may allow independent investigators to conduct secondary assessments on specific safety

issues and to clarify important questions, as it was the case for selective serotonin reuptake inhibitors in the treatment of depression in children (WHITTINGTON *et al.*, 2004). Moreover, analyses of postmarketing spontaneous reports compiled in databases of regulatory authorities, such as the FDA, by other investigators have led to the generation of safety signals and the production of scientific literature, thereby proving to be a good strategy in drug safety monitoring (POWERS and COOK, 2012).

Surprisingly, only three of the 59 safety signals studied, were simultaneously raised and evaluated by all four regulatory agencies assessed in this study: pioglitazone and bladder cancer, combined hormonal contraceptives containing drospirenone and venous thromboembolism and citalopram and QT interval prolongation. The vast majority of these drugs were approved in the countries regulated by the four authorities, with the exception of tinzaparin and ketoprofen containing drugs used topically. Since a given alert, which leads to a further regulatory action, can be considered a safety issue by one agency and not by another agency, similar populations may be at different levels of risk due to regulatory decisions. Moreover, when the same safety issues were simultaneously evaluated by more than one agency, decisions were taken within different time frames, as was the case for proton pump inhibitors and bone fractures or hypomagnesemia, pioglitazone or escitalopram, with a difference of several months. A study conducted by Hirst and colleagues (2006) described some examples of different regulatory actions conducted for the same medicines in different countries - however, in this study discrepancies in label changes were rare.

Although guidelines have been developed to harmonize pharmacovigilance activities worldwide, differences in healthcare systems, regulatory procedures and even in culture may contribute to risk management strategies varying across countries (HIRST *et al.*, 2006; US FOOD AND DRUG ADMINISTRATION, 2013; PFISTERMEISTER *et al.*, 2013). Additionally, surveillance of all approved drugs and their potential adverse reactions in an active way may not be feasible due to restrictions in human resources and/or budget (WYSOWSKI and SWARTZ, 2005; HIRST *et al.*, 2006). Cooperative agreements may be established between regulatory authorities to monitor various activities, including discussions of safety issues, exchange of pharmacovigilance information and collaboration in conducting studies to clarify safety issues (EUROPEAN MEDICINES AGENCY, 2013a; EUROPEAN MEDICINES AGENCY, 2013b; STANG, M.; WYSOWSKI, D.K.; BUTLER-JONES, 1999). Despite guidance and cooperation, differences in safety regulation between major regulatory authorities still exist, such as the discrepancies in drug label updates conducted by FDA and EMA on the cardiovascular safety

of non-selective non-steroids anti-inflammatory drugs - decisions which were based on the same scientific evidence (FURBERG, 2007). As Hirst and colleagues (2006) previously stated, since the methods applied for evaluating benefit/risk ratio may not be comparable, inconsistent regulatory action around the world may be inevitable.

Most of the label changes identified in our study resulted in an update of the Warnings and precautions section, with only two boxed warnings being added and a marketing authorization being suspended. Similar studies on this subject identified more boxed warnings added to labels (MOORE, SINGH and FURBERG, 2012; LESTER *et al.*, 2013). The majority of the drugs included in this study have been marketed for several years almost worldwide and have thus been used to treat millions of people. Recently approved drugs may be more likely to be associated with unknown and serious adverse drug reactions. A previous study found that half of drug withdrawals occur within 2 years after a market authorization has been granted and that half of major label changes (defined as "drug withdrawal" or "black box warning inclusion") occur within 7 years after drug approval (LASSER *et al.*, 2012). Additionally, changes in drug development that have led to important safety issues being taken into consideration may also have led to drug withdrawal due to common causes, such as hepatotoxicity or cardiovascular toxicity, to have become less likely (TEMPLE and HIMMEL, 2002).

This study has a number of limitations. Regulatory agencies other than FDA, Health Canada, EMA and Australian TGA were not searched. Websites posted in languages other than English were not considered, which could have resulted in the exclusion of important information. We analysed safety alerts and communications which included early notices on safety issues and, therefore, some of these continue to be under revision at this time, without as yet any final decision by the authorities; additionally, information on data sources and regulatory actions may not be published in their entirety. Safety signals generated through the analysis of postmarketing spontaneous reports databases were not specifically searched since such information should be confirmed by the authorities due to its uncertainties.

Regulatory authorities continuously review the benefit/risk ratio of a drug throughout its entire life time, taking into account that data sources which are available will differ substantially. During the post-marketing phase, once an AE is possibly associated with drug treatment, regulatory authorities assess the extent to which it may be a threat to public health (MADRE *et al.*, 2006). Postmarketing spontaneous reporting systems have been shown to be a valuable resource by which to identify suspected adverse drug reactions, especially

those which are rare and serious. Harmonization between regulatory authorities of different regions should be the subject of further efforts in order to expedite the decision-making process and to understand the reason(s) for the differences in the length of time involved in the regulatory safety decision process.

V.6. BIBLIOGRAPHY

AHMAD, S.R.; MARKS, N.S.; GOETSCH, R.A. – Spontaneous reporting in the United States. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 91-116.

BULL, J. – US activities in risk management of pharmaceutical products. In: MANN, Ronald, ANDREWS, Elizabeth, Pharmacovigilance, 2nd Ed. Chichester, England: John Wiley & Sons, 2007, ISBN: 978-0-470-01803-3. p. 535-552.

DAL PAN, G.J.; TEMPLE, R. – Balancing transparency and uncertainty. **Arch Intern Med.** 172;1 (2012) 74-75.

DORE, D.D. [et al] – A cohort study of acute pancreatitis in relation to exenatide use. **Diabetes Obes Metab.** 13;6 (2011) 559-66.

DORE, D.D.; SEEGER, J.D.; CHAN, K.A. – Use of a claims-based active drug safety surveillance system to assess the risk of acute pancreatitis with exenatide or sitagliptin compared to metformin or glyburide. **Curr Med Res Opin.** 25;4 (2009) 1019-1027.

EDWARDS, I.R. [et al] – Global Drug Surveillance: The WHO Programme for International Drug Monitoring. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 117-136.

EDWARDS, I.R.; BIRIELL, C. – Harmonisation in Pharmacovigilance. **Drug Saf.** 10;2 (1994) 93-102.

EUROPEAN MEDICINES AGENCY – Monthly reports of the CHMP Pharmacovigilance Working Party. (2014) Available FROM: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/document_listing/document_listing_000198.jsp&mid=WC0b01ac05805a8fd3

EUROPEAN MEDICINES AGENCY – News, press release and public statement archive. (2014) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/landing/news_search_landing_page.jsp&mid=WC0b01ac058004d5c1

EUROPEAN MEDICINES AGENCY – Partners & Networks. Regulators outside the EU. United States. (2013a) Available from:

http://www.ema.europa.eu/ema/index.jsp?curl=pages/partners_and_networks/document_listing/document_listing_000228.jsp&mid=WC0b01ac058003176e

EUROPEAN MEDICINES AGENCY – Partners & Networks. Regulators outside the EU. Canada. (2013b) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/partners_and_networks/document_listing/document_listing_000230.jsp&mid=WC0b01ac0580034f00

FURBERG, C.D. – Decisions by regulatory agencies: are they evidence-based? **Trials**. 8 (2007) 13.

GARG, R.; CHEN, W.; PENDERGRASS, M. – Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. **Diabetes Care.** 33;11 (2010) 2349-2354.

GUYATT, G.H. [et al] – GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. **BMJ**. 336;7650 (2008) 924-926.

HEALTH CANADA – Advisories, warnings and recalls. (2013) Available from: http://www.hc-sc.gc.ca/dhp-mps/advisories-avis/index-eng.php

HIRST, C. [et al] – A call for international harmonization in therapeutic risk management. Pharmacoepidemiol **Drug Saf.** 15;12 (2006) 839-849.

LASSER, K.E. [et al] – Timing for new black box warnings and withdrawals for prescription medications. **JAMA.** 287;17 (2012) 2215-2220.

LESTER, J. [et al] – Evaluation of FDA safety-related drug label changes in 2010. **Pharmacoepidemiol Drug Saf.** 22;3 (2013) 302-305.

MADRE, L. [et al] – Views from Academia, Industry, and Regulatory Agencies. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 63-88.

MOORE, T.J.; SINGH, S.; FURBERG, C.D. – The FDA and new safety warnings. **Arch Intern Med.** 172;1 (2012) 78-80.

PAPANIKOLAOU, P.N.; CHRISTIDI, G.D.; IOANNIDIS, J.P.A. – Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. **CMAJ**. 174;5 (2006) 635-641.

PFISTERMEISTER, B. [et al] – Different indications, warnings and precautions, and contraindications for the same drug - an international comparison of prescribing information for commonly used psychiatric drugs. **Pharmacoepidemiol Drug Saf.** 22;3 (2013) 329-333.

POWERS, A.; COOK, G.E. – Potential safety signals and their significance. **Arch Intern Med.** 172;1 (2012) 72-73.

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

STANG, M.; WYSOWSKI, D.K.; BUTLER-JONES, D. – Incidence of lactic acidosis in metformin users. **Diabetes Care.** 22;6 (1999) 925-927.

TEMPLE, R.J.; HIMMEL, M.H. – Safety of newly approved drugs: implications for prescribing. **JAMA**. 287;17 (2002) 2273-2275.

THERAPEUTIC GOODS ADMINISTRATION – Safety information. Alerts. (2014) Available from: http://www.tga.gov.au/safety/alerts-all-date.htm

THERAPEUTIC GOODS ADMINISTRATION – Safety information. Product recalls. (2014) Available from: http://www.tga.gov.au/safety/recalls-all-date.htm

US FOOD AND DRUG ADMINISTRATION – Byetta (exenatide) injection. Detailed view: safety labelling changes approved by FDA Centre for Drug Evaluation and Research. MedWatch the FDA Safety Information and Adverse Event Reporting Program. (2009) Available from: http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm190571.htm

US FOOD AND DRUG ADMINISTRATION – Drug Safety Communications. (2014) Available from: http://www.fda.gov/Drugs/DrugSafety/ucm199082.htm

US FOOD AND DRUG ADMINISTRATION – Guidance drug safety information - FDA's Communication to Public. Centre for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). (2012) Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM295217.pdf

US FOOD AND DRUG ADMINISTRATION – Guidance for Industry – Warnings and precautions, contraindications, and boxed warning sections of labeling for human prescription drug and biological products – content and format. Centre for Drug Evaluation and Research (CDER),

Center for Biologics Evaluation and Research (CBER). (2011) Available from: http://www.fda.gov/downloads/Drugs/Guidances/ucm075096.pdf.

US FOOD AND DRUG ADMINISTRATION – Guidance, Compliance & Regulatory Information. International Conference on Harmonisation - Safety. (2013) Available from: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065007.htm

US FOOD AND DRUG ADMINISTRATION – The Sentinel Initiative: National Strategy for Monitoring Medical Product Safety. Department of Health and Human Services. (2008) Available from: http://www.fda.gov/downloads/Safety/FDAsSentinelInitiative/UCM124701.pdf

VANDENBROUCKE, J.P.; PSATY, B.C. – Benefits and risks of drug treatments: how combine the best evidence on benefits with the best data about adverse effects. **JAMA**. 300;20 (2008) 2417-2419.

WHITTINGTON, C.J. [et al] – Selective serotonin reuptake inhibitors in childhood depression: systematic review of published versus unpublished data. **Lancet.** 363;9418 (2004) 1341-1345.

WHO COLLABORATION CENTRE FOR DRUG STATISTICS METHODOLOGY – ATC/DDD Index 2014. (2014) Available from http://www.whocc.no/atc_ddd_index/

WOLFE, S.M. – Early signals of harmful drugs. **Arch Intern Med.** 172;1 (2012) 73-74.

WYSOWSKI, D.K.; SWARTZ, L. – Adverse drug event surveillance and drug withdrawals in the United States, 1969-2002: the importance of reported suspected reactions. **Arch Inter Med.** 165;12 (2005) 1363-1369.

V.7. SUPPLEMENTAL DATA V

V.7.1. SUPPLEMENTAL DATA V.1 - WORLDWIDE MOST CONSUMED DRUG CLASSES IN AMBULATORY CARE FROM 2010 UNTIL SEPTEMBER 2012

Supplemental Table V.1 - Worldwide most consumed drug classes in ambulatory care from 2010 until September 2012.

ATC 2nd level Worldwide Ambulatory setting	Units* September 2012	Units* Year 2011	Units* Year 2010
TOTAL	**86.705.920**	**85.303.455**	**81.295.783**
J1 (SYSTEMIC ANTIBACTERIALS)	7.338.442	7.202.647	7.058.254
N2 (ANALGESICS)	6.341.166	6.267.979	6.040.099
A2 (A-ACID A-FLAT A-ULCERANT)	5.399.376	5.217.473	4.763.242
R5 (COUGH,COLD PREPARATIONS)	4.750.842	4.867.359	4.628.154
M1 (ANTIRHEUMATIC SYSTEM)	3.931.491	3.920.386	3.756.272
A11 (VITAMINS)	3.824.783	3.714.156	3.614.190
A10 (DRUGS USED IN DIABETES)	3.005.279	2.865.126	2.569.084
R3 (ANTI-ASTHMA & COPD PROD)	2.743.511	2.772.934	2.606.099
C9 (RENIN-ANGIOTEN SYS AGENT)	2.502.718	2.428.457	2.242.174
A7 (A-DIAR ORAL ELEC+A-INFLA)	2.339.231	2.222.082	2.128.853
N5 (PSYCHOLEPTICS)	2.112.611	2.152.871	2.130.181
H2 (SYSTEMIC CORTICOSTEROIDS)	1.953.957	1.927.224	1.859.051
S1 (OPHTHALMOLOGICALS)	1.941.345	1.917.163	1.894.804
D7 (TOPICAL CORTICOSTEROIDS)	1.660.655	1.589.325	1.476.307
A3 (GAST-INTEST DISORD DRUG)	1.557.979	1.570.617	1.474.761
R6 (ANTIHISTAMINES SYSTEMIC)	1.429.452	1.419.120	1.349.397
R1 (NASAL PREPARATIONS)	1.405.983	1.389.094	1.332.254
C8 (CALCIUM ANTAGONISTS)	1.401.255	1.371.635	1.307.215
N6 (PSYCHOANALEPTICS)	1.374.850	1.342.811	1.261.624
B1 (ANTITHROMBOTIC AGENTS)	1.355.129	1.342.219	1.274.782
C7 (BETA BLOCKING AGENTS)	1.352.113	1.317.395	1.238.400
C10 (LIP.REG./ANTI-ATH. PREPS)	1.336.965	1.305.105	1.218.968
P1 (ANTIPROTOZOALS & ANTHELMIN)	1.268.085	1.275.381	1.278.451
G3 (SEX HORMONES-SYSTEMIC)	1.235.709	1.216.392	1.162.638
N3 (ANTI-EPILEPTICS)	1.188.892	1.128.611	1.029.457
M2 (ANTIRHEUMATICS TOPICAL)	1.017.346	984.034	942.144
B3 (ANTIANAEMICS)	1.002.198	987.989	937.067
V6 (GENERAL NUTRIENTS)	906.938	880.395	822.031

* Thousands of units.

V.7.2. SUPPLEMENTAL DATA V.2 - REFERENCES FROM STUDIES PRESENTED AT TABLE V.1

1. DIAV-CITRIN, O. [et al] – Paroxetine and fluoxetine in pregnancy: a prospective, multicentre, controlled, observational study. **Br J Clin Pharmacol.** 66;5 (2008) 695-705.

2. ELI LILLY AND COMPANY CLINICAL TRIAL REGISTRY – Results Observational/Non-interventional studies. (2013) Available from: http://www.lillytrials.com/nonintstudies/result_files/13947.pdf

3. VESTERGAARD, P.; REJNMARK, L.; MOSEKILDE, L. – Proton pump inhibitors, histamine H2 receptor antagonists, and other antacid medications and the risk of fracture. **Calcif Tissue Int.** 79;2 (2006) 76-83.

4. YANG, Y.X. [et al] – Long-term proton pump inhibitor therapy and risk of hip fracture. **JAMA**. 296;24 (2006) 2947-2953.

5. TARGOWNIK, L.E. [et al] – Use of proton pump inhibitors and risk of osteoporosis-related fractures. **CMAJ**. 179;4 (2008) 319-326.

6. CORLEY, D.A. [et al] – Proton pump inhibitors and histamine-2 receptor antagonists are associated with hip fractures among at-risk patients. **Gastroenterology.** 139;1 (2010) 93-101.

7. GRAY, S.L. [et al] – Proton pump inhibitor use, hip fracture, and change in bone mineral density in postmenopausal women. **Arch Intern Med.** 170;9 (2010) 765-771.

8. YU, E.W. [et al] – Acid-suppressive medications and risk of bone loss and fracture in older adults. **Calcif Tissue Int.** 83;4 (2008) 251-259.

9. KAYE, J.A.; JICK, H. – Proton pump inhibitor use and risk of hip fractures in patients without major risk factors. **Pharmacotherapy** 28;8 (2008) 951-959.

10. TARGOWNIK, L.E. [et al] – Proton-pump inhibitor use is not associated with osteoporosis or accelerated bone mineral density loss. **Gastroenterology.** 138;3 (2010) 896-904.

11. DE VRIES, F. [et al] – Fracture risk in patients receiving acid suppressant medication alone and in combination with bisphosphonates. **Osteoporos Int.** 20;12 (2009) 1989-1998.

12. ROUX, C. [et al] – Increase in vertebral fracture risk in postmenopausal women using omeprazole. **Calcif Tissue Int.** 84;1 (2009) 13-19.

13. KWOK, C.S.; YEONG, J.K.; LOKE, Y.K. – Meta-analysis: risk of fractures with acid-suppressing medication. **Bone.** 48;4 (2011) 768-776.

14. EOM, C.S. – Use of acid-suppressive drugs and risk of fracture: a meta-analysis of observational studies. **Ann Fam Med.** 9;3 (2011) 257-267.

15. RIDKER, P.M. [et al] – Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. **N Engl J Med.** 359;21 (2008) 2195-2207.

16. SIPAHI, I. – Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. **Lancet Oncol.** 11;7 (2010) 627-636.

17. BANGALORE, S. [et al] – Antihypertensive drugs and risk of cancer: network meta-analyses and trial sequential analyses of 324,168 participants from randomized trials. **Lancet Oncol.** 12;1 (2011) 65-82.

18. THE ARB TRIALISTS COLLABORATION – Effects of telmisartan, irbesartan, valsartan, and candesartan, and losartan on cancers in 15 trials. **J Hypertens.** 29;4 (2011) 623-635.

19. PASTERNAK, B. – Use of angiotensin receptor blockers and the risk of cancer. **Circulation.** 123;16 (2011) 1729-1736.

20. LAL, Y.; ASSIMACOPOULOS, A.P. – Two cases of daptomycin-induced eosinophilic pneumonia and chronic pneumonitis. **Clin Infect Dis.** 50;5 (2010) 737-740.

21. HAYES, D. JR, ANSTEAD, M.I.; KUHN, R.J. – Eosinophilic pneumonia induced by daptomycin. **J Infect.** 54;4 (2007) e211-e213.

22. MILLER, B.A. [et al] – Acute eosinophilic pneumonia secondary to daptomycin: a report of three cases. **Clin Infect Dis.** 50;11 (2010) e63-e68.

23. KAKISH, E. [et al] – Acute respiratory failure due to daptomycin induced eosinophilic pneumonia. **Respir Med CME.** 1;3 (2008) 235-237.

24. DORMANDY, J.A. [et al] – Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. **Lancet.** 366;9493 (2005) 1279-1289.

25. LEWIS, J.D. [et al] – Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. **Diabetes Care.** 34;4 (2011) 916-922.

26. NEUMANN, A. [et al] – Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. **Diabetologia.** 55;7 (2012) 1953-1962.

27. AZOULAY, L. [et al] – The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. **BMJ**. 344 (2012) e3645 doi:10.1136/bmj.e3645.

28. LEIZOROVICZ, A. [et al] – Safety profile of tinzaparin versus subcutaneous unfractionated heparin in elderly patients with impaired renal function treated for acute deep vein thrombosis: the Innohep® in Renal Insufficiency Study (IRIS). **Thromb Res.** 128;1 (2011) 27-34.

29. HEDEROS, C.A. – Neuropsychologic changes and inhaled corticosteroids. **J Allergy Clin Immunol.** 114 (2004) 451-452.

30. POKLADNIKOVA, J. – Intranasally administered corticosteroids and neuropsychiatric disturbances: a review of the international pharmacovigilance programme of the World Health Organization. **Ann Allergy Asthma Immunol.** 101;1 (2008) 67-73.

31. STUART, F.A.; SEGAL, T.Y.; KEADY, S. – Adverse psychological effects of corticosteroids in children and adolescents. **Arch Dis Child.** 90;5 (2005) 500-506.

32. TAVASSOLI, N. [et al] – Psychiatric adverse drug reactions to glucocorticoids in children and adolescents: a much higher risk with elevated doses. **Br J Clin Pharmacol.** 66;4 (2008) 566-567.

33. DE VRIES, T.W. [et al] – Reported adverse drug reactions during the use of inhaled steroids in children with asthma in the Netherlands. **Eur J Clin Pharmacol.** 62;5 (2006) 343–346.

34. DE VRIES, T.W.; VAN ROON, E.N.; DUIVERMAN, E.J. – Inhaled corticosteroids do not affect behaviour. **Acta Paediatr.**97;6 (2008) 786-789.

35. BROEREN, M.A. [et al] – Hypomagnesium induced by several proton-pump inhibitors. **Ann Intern Med.** 151;10 (2009) 755-756.

36. CUNDY, T.; DISSANAYAKE, A. – Severe hypomagnesemia in long-term users of proton-pump inhibitors. **Clinical Endocrinology.** 69;2 (2008) 338-341.

37. EPSTEIN, M.; MCGRATH, S.; LAW, F. – Proton-pump inhibitors and hypomagnesemic hypoparathyroidism. **N Engl J Med.** 355;17 (2006) 1834-1836.

38. HOORN, E.J. [et al] – A case series of proton pump inhibitor–induced hypomagnesemia. **Am J Kidney Dis.** 56;1 (2010) 112-116.

39. KUIPERS, M.T.; THANG, H.D.; ARNTZENIUS, A.B. – Hypomagnesaemia due to use of proton pump inhibitors—a review. **Neth J Med.** 67;5 (2009) 169-172.

40. METZ, D.C. [et al] – Effects of esomeprazole on acid output in patients with Zollinger-Ellison syndrome or idiopathic gastric acid hypersecretion. **Am J Gastroenterol.** 102;12 (2007) 2648-2654.

41. SHABAJEE, N. [et al] – Omeprazole and refractory hypomagnesemia. **BMJ**. 337;7662 (2008) 173-175.

42. MACKAY, J.D.; BLADON, P.T. – Hypomagnesaemia due to proton-pump inhibitor therapy: a clinical case series. **QJM**. 103;6 (2010) 387-395.

43. SWAMINATHAN, K.; WILSON, J. – Elusive cause of hypomagnesaemia. **BMJ**. 343 (2011) d5087 doi: 10.1136/bmj.d5087.

44. PARKIN, L. [et al] – Risk of venous thromboembolism in users of oral contraceptives containing drospirenone or levonorgestrel: nested case-control study based on UK General Practice Research Database. **BMJ**. 342 (2011) d2139 doi:10.1136/bmj.d2139.

45. JICK, S.S.; HERNANDEZ, R.K. – Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. **BMJ**. 342 (2011) d2151 doi:10.1136/bmj.d2151.

46. LIDEGAARD, Ø. [et al] – Hormonal contraception and risk of venous thromboembolism: national follow-up study. **BMJ**. 339 (2009) b2890 doi:10.1136/bmj.b2890.

47. VAN HYLCKAMA, V. [et al] – The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. **BMJ**. 339 (2009) b2921. doi: 10.1136/bmj.b2921.

48. DINGER, J.C.; HEINEMANN, L.A.; KÜHL-HABICH, D. – The safety of a drospirenone-containing oral contraceptive: final results from the European Active Surveillance Study on oral contraceptives based on 142,475 women-years of observation. **Contraception.** 75;5 (2007) 344-354.

49. SEEGER, J.D. [et al] – Risk of thromboembolism in women taking ethinylestradiol/drospirenone and other oral contraceptives. **Obstet Gynecol.** 110;3 (2007) 587-593.

50. DINGER, J. [et al] – Risk of venous thromboembolism and the use of dienogest- and drospirenone-containing oral contraceptives: results from a German case-control study. **J Fam Plann Reprod Health Care.** 36;3 (2010) 123-129.

51. MEADOR, K.J. [et al] – Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. **N Engl J Med.** 360;16 (2009) 1597-1605.

52. GAILY, E. [et al] – Normal intelligence in children with prenatal exposure to carbamazepine. **Neurology.** 62;1 (2004) 28-32.

53. ADAB, N.; JACOBY, A.D.; CHADWICK, D. – Additional educational needs of children born to mothers with epilepsy. **J Neuro Neurosurg Psychiatry.** 70;1 (2001) 15-21.

54. ADAB, N. [et al] – The longer term outcome of children born to mothers with epilepsy. **J Neurol Neurosurg Psychiatry.** 75;11 (2004) 1575-1583.

55. COLE, Z.A.; DENNISON, E.M.; COPPER, C. – Osteoporosis epidemiology update. **Curr Rheumatol Rep.** 10;2 (2008) 92-96.

56. JOHNELL, O.; KANIS, J.A. – An estimate of the world-wide prevalence and disability associated with osteoporotic fractures. **Osteoporos Int.** 17;12 (2006) 1726-1733.

57. JETTÉ, N. – Association of antiepileptic drugs with nontraumatic fractures: a population based analysis. **Arch Neurol.** 68;1 (2011) 107-112.

58. VESTERGAARD, P.; REJNMARK, L.; MOSEKILDE, L. – Fracture risk associated with use of antiepileptic drugs. **Epilepsia.** 45;11 (2004) 1330-1337.

59. AKIN, R. [et al] – Evaluation of bone mineral density in children receiving antiepileptic drugs. **Pediatr Neurol.** 19;2 (1998) 129-131.

60. ANDRESS, D.L. [et al] – Antiepileptic drug-induced bone loss in young male patients who have seizures. **Arch Neurol.** 59;5 (2002) 781-786.

61. BELL, R.D. [et al] – Effect of phenytoin on bone and vitamin D metabolism. **Ann Neurol.** 5;4 (1979) 374-378.

62. BOLUK, A. [et al] – The effect of valproate on bone mineral density in adult epileptic patients. **Pharmacol Res.** 50;1 (2004) 93-97.

63. CHOU, I.J. [et al] – Evaluation of bone mineral density n children receiving carbamazepine or valproate monotherapy. **Acta Paediatr Taiwan.** 48;6 (2007) 317-322.

64. CHUNG, S.; AHN, C. – Effects of anti-epileptic drug therapy on bone mineral density in ambulatory epileptic children. **Brain Dev.** 16;5 (1994) 382-385.

65. ECEVIT, C. [et al] – Effect of carbamazepine and valproate on bone mineral density. **Pediatr Neurol.** 31;4 (2004) 279-282.

66. ENSRUD, K.E. [et al] – Antiepileptic drug use increases rates of bone loss in older women: a prospective study. **Neurology.** 62;11 (2004) 2051-2057.

67. FARHAT, G. [et al] – Effect of antiepileptic drugs on bone density in ambulatory patients. **Neurology.** 58;9 (2002) 1348-1353.

68. FELDKAMP, J. [et al] – Long-term anticonvulsant therapy leads to low bone mineral density - evidence for direct drug effects of phenytoin and carbamazepine on human osteoblast-like cells. **Exp Clin Endocrinol Diabetes**. 108;1 (2000) 37-43.

69. GUO, C.Y.; RONEN, G.M.; ATKINSON, S.A. – Long-term valproate and lamotrigine treatment may be a marker for reduced growth and bone mass in children with epilepsy. **Epilepsia.** 42;9 (2001) 1141-1147.

70. HAHN, T.J.; HALSTEAD, L.R.; DEVIVO, D.C. – Disordered mineral metabolism produced by ketogenic diet therapy. **Calcif Tissue Int.** 28;1 (1979) 17-22.

71. KAFALI, G.; ERSELCAN, T.; TANZER, F. – Effect of Antiepileptic Drugs on Bone Mineral Density in Children between Ages 6 and 12 years. **Clin Pediatr (Phila).** 38;2 (1999) 93-98.

72. KUBOTA, F. [et al] – Bone mineral density of epileptic patients on long-term antiepileptic drug therapy: a quantitative digital radiography study. **Epilepsy Res.** 33;2-3 (1999) 93-97.

73. KULAK, C.A. [et al] – Bone mineral density and serum levels of 25 OH vitamin D in chronic users of antiepileptic drugs. **Arq Neuropsiquiatr.** 62;4 (2004) 940-948.

74. KUMANDAS, S. [et al] – Effect of carbamazepine and valproic acid on bone mineral density, IGF-I and IGFBP-3. **J Pediatr Endocrinol Metab.** 19;4 (2006) 529-534.

75. MOSEKILDE, L.; MELSEN, F. – Anticonvulsant osteomalacia determined by quantitative Analysis of bone changes. Population study and possible risk factors. **Acta Med Scand.** 199;5 (1976) 349-355.

76. NILSSON, O.S. [et al] – Fracture incidence and bone disease in epileptics receiving long-term anticonvulsant drug treatment. **Arch Orthop Trauma Surg.** 105;3 (1986) 146-149.

77. NISHIYAMA, S.; KUWAHARA, T.; MATSUDA, I. – Decreased bone density in severely handicapped children and adults, with reference to the influence of limited mobility and anticonvulsant medication. **Eur J Pediatr.** 144;5 (1986) 457-463.

78. ONER, N. [et al] – Bone mineral metabolism changes in epileptic children receiving valproic acid. **J Paediatr Child Health.** 40;8 (2004) 470-473.

79. PACK, A.M. [et al] – Bone mass and turnover in women with epilepsy on antiepileptic drug monotherapy. **Ann Neurol.** 57;2 (2005) 252-257.

80. PACK, A.M. [et al] – Bone mineral density changes after one year of antiepileptic drug treatment in women with epilepsy. **Epilepsia.** 45;suppl 7 (2004) 187.

81. PACK, A.M. [et al] – Bone mineral density in an outpatient population receiving enzyme-inducing antiepileptic drugs. **Epilepsy Behav.** 4;2 (2003) 169-174.

82. PETTY, S.J. [et al] – Effect of antiepileptic medication on bone mineral measures. **Neurology.** 65;9 (2005) 1358-1363.

83. PLUSKIEWICZ, W.; NOWAKOWSKA, J. – Bone status after long-term anticonvulsant therapy in epileptic patients: evaluation using quantitative ultrasound of calcaneus and phalanges. **Ultrasound Med Biol.** 23;4 (1997) 553-558.

84. RIEGER-WETTENGL, G. [et al] – Analysis of the musculoskeletal system in children and adolescents receiving anticonvulsant monotherapy with valproic acid or carbamazepine. **Pediatrics.** 108;6 (2001) E107.

85. RODBRO, P.; CHRISTIANSEN, C.; LUND, M. – Development of anticonvulsant osteomalacia in epileptic patients on phenytoin treatment. **Acta Neurol Scand.** 50;4 (1974) 527-532.

86. SATO, Y. [et al] – Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy. **Neurology.** 57;3 (2001) 445-449.

87. SHETH, R.D. [et al] – Effect of carbamazepine and valproate on bone mineral density. **J Peadiatr.** 127;2 (1995) 256-262.

88. STEPHEN, L.J. [et al] – Bone density and antiepileptic drugs: a case-controlled study. **Seizure.** 8;6 (1999) 339-342.

89. TEKGUL, H. [et al] – Bone mineral status in pediatric outpatients on antiepileptic drug monotherapy. **J Child Neurol.** 21;5 (2006) 411-414.

90. TRAVERSO, A. [et al] – The influence of phenobarbital, carbamazepine, and diphenylhydantoin on bone mineral content in epileptic patients on chronic treatment. **Ital J Miner Elect M.** 10;1 (1996) 19-22.

91. TSUKAHARA, H. [et al] – Bone mineral status in ambulatory pediatric patients on long-term anti-epileptic drug therapy. **Pediatr Int.** 44;3 (2002) 247-253.

92. VÄLIMÄKI, M.J. [et al] – Bone mineral density measured by dual-energy x-ray absorptiometry and novel markers of bone formation and resorption in patients on antiepileptic drugs. **J Bone Miner Res.** 9;5 (1994) 631-637.

93. VESTERGAARD, P. – Epilepsy, osteoporosis and fracture risk – a meta-analysis. **Acta Neurol Scand.** 112;5 (2005) 277-286.

94. SHETH, R.D.; HERMANN, B.P. – Bone mineral density with lamotrgine monotherapy for epilepsy. **Pediatr Neurol.** 37;4 (2007) 250-254.

95. TIMPERLAKE, R.W. [et al] – Effects of anticonvulsant drug therapy on bone mineral density in a pediatric population. **J Pediatr Orthop.** 8;4 (1988) 467-470.

96. RENOUX, C.; DELL'ANIELLO, S.; SUISSA, S. – Hormone replacement therapy and the risk of venous thromboembolism: a population-based study. **J Thromb Haemost.** 8;5 (2010) 979-986.

97. DE VRIES CS.; BROMLEY, S.E.; FARMER, R.D.T. – Myocardial infarction risk and hormone replacement: differences between products. **Maturitas.** 53;3 (2006) 343-350.

98. BERAL, V.; MILLION WOMEN STUDY COLLABORATORS – Breast cancer and hormone-replacement therapy in the Million Women Study. **Lancet.** 362;9382 (2003) 419-427.

99. CONNOLLY, S.J. [et al] – Dabigatran versus warfarin in patients with atrial fibrillation. **N Engl J Med.** 361;12 (2009) 1139-51.

100. SHEPHERD, J. [et al] – Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. **Lancet.** 360;9346 (2002) 1623-1630.

101. SATTAR, N. [et al] – Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. **Lancet.** 375;9716 (2010) 735-742.

102. FREEMAN, D.J. [et al] – Pravastatin and the Development of Diabetes Mellitus: Evidence for a Protective Treatment Effect in the West of Scotland Coronary Prevention Study. **Circulation.** 103;3 (2001) 357-362.

103. KOSTAPANOS, M.S. [et al] – Do statins beneficially or adversely affect glucose homeostasis? **Curr Vasc Pharmacol.** 8;5 (2010) 612-631.

104. SABATINE, M.S. – High-dose atorvastatin associated with worse glycemic control: a PROVE-IT TIMI 22 substudy. **Circulation.** 110;suppl I (2004) S834.

105. RAJPATHAK, S.N. [et al] – Statin therapy and risk of developing type 2 diabetes: a meta-analysis. **Diabetes Care.** 32;10 (2009) 1924-1929.

106. SUKHIJA, R. [et al] – Effect of statins on fasting plasma glucose in diabetic and nondiabetic patients. **J Investig Med.** 57;3 (2009) 495-499.

107. KOH, K.K. [et al] – Atorvastatin causes insulin resistance and increases ambient glycemia in hypercholesterolemic patients. **J Am Coll Cardiol.** 55;12 (2010) 1209-1216.

108. THONGTANG, N. [et al] – Effects of maximal atorvastatin and rosuvastatin treatment on markers of glucose homeostasis and inflammation. **Am J Cardiol.** 107;3 (2011) 387-392.

109. MILLS, E.J. [et al] – Efficacy and safety of statin treatment for cardiovascular disease: a network meta-analysis of 170,255 patients from 76 randomized trials. **QJM**. 104;2 (2011) 109-124.

110. CULVER, A.L. [et al] – Statin use and risk of diabetes mellitus in postmenopausal women in the Women's Health Initiative. **Arch Intern Med.** 172;2 (2012) 144-152.

111. AL-TUREIHI, F.I.J. [et al] – Albumin, length of stay, and proton pump inhibitors: key factors in Clostridium difficile-associated disease in nursing home patients. **J Am Med Dir Assoc.** 6;2 (2005) 105-108.

112. CUNNINGHAM, R. [et al] – Proton pump inhibitors as a risk factor for Clostridium difficile diarrhoea. **J Hosp Infect.** 54 (2003) 243-245.

113. DIAL, S. [et al] – Risk of Clostridium difficile diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. **CMAJ**. 171;1 (2004) 33-38.

114. DIAL, S. [et al] – Use of gastric acid-suppressive agents and the risk of community-acquired Clostridium difficile-associated disease. **JAMA**. 294;23 (2005) 2989-2995.

115. MUTO, C. [et al] – A large outbreak of Clostridium difficile associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. **Infect Control Hosp Epidemiol.** 26;3 (2005) 273-280.

116. DIAL, S. – Proton pump inhibitor use and risk of community-acquired Clostridium difficile-associated disease defined by prescription for oral vancomycin therapy. **CMAJ**. 175;7 (2006) 745-748.

117. DIAL, S. [et al] – Patterns of antibiotic use and risk of hospital admission for Clostridium difficile infection among elderly people in Quebec. **CMAJ**. 179;8 (2008) 767-772.

118. AKHTAR, A.J.; SHAHEEN, M. – Increasing incidence of Clostridium difficile-associated diarrhea in African-American and Hispanic patients: association with the use of proton pump inhibitor therapy. **J Natl Med Assoc.** 99;5 (2007) 500-504.

119. ASEERI, M. [et al] – Gastric acid suppression by proton pump inhibitors as a risk factor for Clostridium difficile-associated diarrhea in hospitalized patients. **Am J Gastroenterol.** 103;9 (2008) 2308-2313.

120. CADLE, R.M. [et al] – Association of proton-pump inhibitors with outcomes in Clostridium difficile colitis. **Am J Health Syst Pharm.** 64;22 (2007) 2359-2363.

121. DALTON, B.R. [et al] – Proton pump inhibitors increase significantly the risk of Clostridium difficile infection in a low-endemicity, non-outbreak hospital setting. **Aliment Pharmacol Ther.** 29;6 (2009) 626-634.

122. DUBBERKE, E.R. [et al] – Clostridium difficile-associated disease in a setting of endemicity: identification of novel risk factors. **Clin Infect Dis.** 45;12 (2007) 1543-1549.

123. HOWELL, M.D. [et al] – Iatrogenic gastric acid suppression and the risk of nosocomial Clostridium difficile infection. **Arch Intern Med.** 170;9 (2010) 784-790.

124. JANARTHANAN, S. [et al] – A meta-analysis of 16 observational studies on proton pump inhibitor use and risk of Clostridium difficile associated diarrhea. **Am J Gastroenterol.** 105;Suppl 1 (2010) S378.

125. JAYATILAKA, S. [et al] – Clostridium difficile infection in an urban medical center: five-year analysis of infection rates among adult admissions and association with the use of proton pump inhibitors. **Ann Clin Lab Sci.** 37;3 (2007) 241-247.

126. KAZAKOVA, S.V. [et al] – A hospital outbreak of diarrhea due to an emerging epidemic strain of Clostridium difficile. **Arch Intern Med.** 166;22 (2006) 2518-2524.

127. KIM, J.W. [et al] – Proton pump inhibitors as a risk factor for recurrence of Clostridium-difficile-associated diarrhea. **World J Gastroenterol.** 16;28 (2010) 3573-3577.

128. LEONARD, J.; MARSHALL, J.K.; MOAYYEDI, P. – Systematic review of the risk of enteric infection in patients taking acid suppression. **Am J Gastroenterol.** 102;9 (2007) 2047-2056.

129. LINSKY, A. [et al] – Proton pump inhibitors and risk for recurrent Clostridium difficile infection. **Arch Intern Med.** 170;9 (2010) 772-778.

130. TURCO, R. [et al] – Proton pump inhibitors as a risk factor for paediatric Clostridium difficile infection. **Aliment Pharmacol Ther.** 31;7 (2010) 754-759.

131. YEARSLEY, K.A. [et al] – Proton pump inhibitor therapy is a risk factor for Clostridium difficile-associated diarrhoea. **Aliment Pharmacol Ther.** 24;4 (2006) 613-619.

132. PEPIN, J. [et al] – Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile-associated diarrhea: a cohort study during an epidemic in Quebec. **Clin Infect Dis.** 41;9 (2005) 1254-1260.

133. SHAH, S. [et al] – Gastric acid suppression does not promote clostridial diarrhoea in the elderly. **QJM**. 93;3 (2000) 175-181.

134. LOO, V.G. [et al] – A predominantly clonal multi-institutional outbreak of Clostridium diffcile-associated diarrhea with high morbidity and mortality. **N Engl J Med.** 353 (2005) 2442-2449.

135. BEAULIEU, M. [et al] – Risk of Clostridium difficile associated disease among patients receiving proton-pump inhibitors in a Quebec medical intensive care unit. **Infect Control Hosp Epidemiol.** 28;11 (2007) 1305-1307.

136. LOWE, D.O. [et al] – Proton pump inhibitors and hospitalization for Clostridium difficile-associated disease: a population-based study. **Clin Infect Dis.** 43;10 (2006) 1272-1276.

137. ORSI, A.; SHERMAN, O.; WOLDESELASSIE, Z. – Simvastatin-associated memory loss. **Pharmacotherapy.** 21;6 (2001) 767-769.

138. PARKER, B.A. [et al] – Changes in memory function and neuronal activation associated with atorvastatin therapy. **Pharmacotherapy**. 30;6 (2010) 236e-240e.

139. WAGSTAFF, L.R. [et al] – Statin-associated memory loss: analysis of 60 case reports and review of the literature. **Pharmacotherapy.** 23;7 (2003) 871-880.

140. EVANS, M.A.; GOLOMB, B.A. – Statin-associated adverse cognitive effects: survey results from 171 patients. **Pharmacotherapy.** 29;7 (2009) 800-811.

141. MULDOON, M.F. [et al] – Effects of lovastatin on cognitive function and psychological well-being. **Am J Med.** 108;7 (2000) 538-546.

142. MULDOON, M.F. [et al] – Randomized trial of the effects of simvastatin on cognitive functioning in hypercholesterolemic adults. **Am J Med.** 117;11 (2004) 823-829.

143. TROMPET, S. [et al] – Pravastatin and cognitive function in the elderly. Results of the PROSPER study. **J Neurol.** 257;1 (2010) 85-90.

144. FELDMAN, H.H. [et al] – Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. **Neurology.** 74;12 (2010) 956-964.

145. ZHOU, B.; TERAMUKAI, S.; FUKUSHIMA, M. – Prevention and treatment of dementia or Alzheimer's disease by statins: a meta-analysis. **Dement Geriatr Cogn Disord.** 23;3 (2007) 194-201.

146. BENITO-LEÓN, J. [et al] – Statins and cognitive functioning in the elderly: a population-based study. **J Alzheimers Dis.** 21;1 (2010) 95-102.

147. BETTERMANN, K. [et al] – Statins, risk of dementia, and cognitive function: secondary analysis of the ginkgo evaluation of memory study. **J Stroke Cerebrovasc Dis.** 21;6 (2012) 436-444.

148. BEYDOUN, M.A. [et al] – Statins and serum cholesterol's associations with incident dementia and mild cognitive impairment. **J Epidemiol Community Health.** 65;11 (2011) 949-957.

149. ZAMRINI, E.; MCGWIN, G.; ROSEMAN, J.M. – Association between statin use and Alzheimer's disease. **Neuroepidemiology.** 23;1-2 (2004) 94-98.

150. ZANDI, P.P. [et al] – Do statins reduce risk of incident dementia and Alzheimer disease? The Cache County Study. **Arch Gen Psychiatry.** 62;2 (2005) 217-224.

151. SHORVON, S.; TOMSON, T. – Sudden unexpected death in epilepsy. **Lancet.** 378;9808 (2011) 2028-2038.

152. NASHEF, L. [et al] – Unifying the definitions of sudden unexpected death in epilepsy. **Epilepsia.** 53;2 (2012) 227-233.

153. AURLIEN, D.; TAUBOLL, E.; GJERSTAD, L. – Lamotrigine in idiopathic epilepsy: increased risk of cardiac death?. **Acta Neurol Scand.** 115;3 (2007) 199-203.

154. AURLIEN, D. [et al] – Increased incidence of sudden unexpected death in epilepsy (SUDEP) with lamotrigine in Rogaland County, Norway. AES 64th Annual Meeting and 3rd Biennial North American Regional Epilepsy Congress. AES Abstracts. Supplement, Vol. 11 Num. 1. (2010) Available from: http://www.aesnet.org/files/dmfile/Volume11Supplement1Abstracts2.pdf

155. HESDORFFER, D.C. [et al] – Combined analysis of risk factors for SUDEP. **Epilepsia.** 52;6 (2011) 1150-1159.

156. HESDORFFER, D.C. [et al] – Do antiepileptic drugs or generalized tonic-clonic seizure frequency increase SUDEP risk?: a combined analysis. **Epilepsia.** 53;2 (2012) 249-252.

157. RYVLIN, P.; CUCHERAT, M.; RHEIMS, S. – Risk of sudden unexpected death in epilepsy in patients given adjunctive antiepileptic treatment for refractory seizures: a meta-analysis of placebo-controlled randomised trials. **Lancet Neurol.** 10;11 (2011) 961-968.

158. GRACE, J.B.; THOMPSON, P. – Neuroleptic malignant like syndrome in two patients on cholinesterase inhibitors. **Int J Geriatr Psychiatry.** 21;2 (2006) 193-194.

159. WARWICK, T.C. [et al] – Neuroleptic malignant syndrome variant in a patient receiving donepezil and olanzapine. **Nat Clin Pract Neurol.** 4;3 (2008) 170-174.

160. OHKOSHI, N. [et al] – Neuroleptic malignant-like syndrome due to donepezil and maprotiline. **Neurology.** 60;6 (2003) 1050-1051.

161. MATSUMOTO, T. [et al] – Neuroleptic malignant syndrome induced by donepezil. **Int J Neuropsychopharmacol.** 7;1 (2004) 101-103.

162. VAN DER MAAREL-WIERINK, C.D. [et al] – Risk factors for aspiration pneumonia in frail older people: a systematic literature review. **J Am Med Dir Assoc.** 12;5 (2001) 344-354.

163. GULMEZ, S.E. [et al] – Use of proton pump inhibitors and the risk of community-acquired pneumonia: a population-based case-control study. **Arch Intern Med.** 167;9 (2007) 950-955.

164. LAHEIJ, R.J. [et al] – Risk of community acquired pneumonia and use of gastric acid suppressive drugs. **J Am Med Assoc.** 292;16 (2004) 1955-1960.

165. MARCINIAK, C. [et al] – Examination of selected clinical factors and medication use as risk factors for pneumonia during stroke rehabilitation: a case-control study. **Am J Phys Med Rehabil.** 88;1 (2009) 30-38.

166. MEIJVIS, S.C. [et al] – Microbial evaluation of proton-pump inhibitors and the risk of pneumonia. **Eur Respir J.** 38;5 (2011) 1165-1172.

167. MYLES, P.R. [et al] – Risk of community-acquired pneumonia and the use of statins, ace inhibitors and gastric acid suppressants: a population-based case-control study. **Pharmacoepidemiol Drug Saf.** 18;4 (2009) 269-275.

168. RODRIGUEZ, L.A. [et al] – Acid suppressive drugs and community-acquired pneumonia. **Epidemiology.** 20;6 (2009) 800-806.

169. SARKAR, M.; HENNESSY, S.; YANG, Y.X. – Proton-pump inhibitor use and the risk for community-acquired pneumonia. **Ann Intern Med.** 149;6 (2008) 391-398.

170. CANANI, R.B. [et al] – Therapy with gastric acidity inhibitors increases the risk of acute gastroenteritis and community acquired pneumonia in children. **Pediatrics.** 117;5 (2006) e817-e820.

171. HERZIG, S.J. [et al] – Acid-suppressive medication use and the risk for hospital-acquired pneumonia. **J Am Med Assoc.** 301;20 (2009) 2120-2128.

172. ROUGHEAD, E.E. [et al] – Proton pump inhibitors and the risk of antibiotic use and hospitalisation for pneumonia. Med J Aust. 190;3 (2009) 114-116.

173. EOM, C.S. [et al] – Use of acidsuppressive drugs and risk of pneumonia: a systematic review and meta-analysis. **Can Med Assoc J.** 183;3 (2011) 310-319.

174. GIULIANIO, C.; WILHELM, S.M.; KALE-PRADHAN, P.B. – Are proton pump inhibitors associated with the development of community-acquired pneumonia?: a meta-analysis. **Expert Rev Clin Pharmacol.** 5;3 (2012) 337-344.

175. JOHNSTONE, J.; NERENBERG, K.; LOEB, M. – Meta-analysis: proton pump inhibitor use and the risk of community-acquired pneumonia. **Aliment Pharmacol Ther.** 31;11 (2010) 1165-1177.

176. LAHEIJ, R. [et al] – Gastric acid suppressive therapy and community-acquired pneumonia, etiology and outcome. **Gastroenterology.** 140;5,Suppl 1 (2011) S391.

177. MIANO, T.A. [et al] – Nosocomial pneumonia risk and stress ulcer prophylaxis: a comparison of pantoprazole vs ranitidine in cardiothoracic surgery patients. **Chest**. 136;2 (2009) 440-447.

178. KASUYA, Y. [et al] – Ventilator associated pneumonia in critically ill stroke patients: frequency, risk factors, and outcomes. **J Crit Care.** 26;2 (2011) 273-279.

179. DUBLIN, S. [et al] – Use of proton pump inhibitors and H2 blockers and risk of pneumonia in older adults: a population-based case-control study. **Pharmacoepidemiol Drug Saf.** 19;8 (2010) 792-802.

180. EURICH, D.T. [et al] – Recurrent community-acquired pneumonia in patients starting acid-suppressing drugs. **Am J Med.** 123;1 (2010) 47-53.

181. GAU, J.T. [et al] – Pharmacotherapy and the risk for community-acquired pneumonia. **BMC Geriatr.** 10 (2010) 45 doi:10.1186/1471-2318-10-45.

182. BEAULIEU, M. [et al] – Do proton-pump inhibitors increase the risk for nosocomial pneumonia in a medical intensive care unit?. **J Crit Care.** 23;4 (2008) 513-518.

183. MALLOW, S. [et al] – Do proton pump inhibitors increase the incidence of nosocomial pneumonia and related infectious complications when compared with histamine-2 receptor antagonists in critically ill trauma patients?. **Curr Surg.** 61;5 (2004) 452-458.

184. REDELMEIER, D.A. [et al] – Postoperative pneumonia in elderly patients receiving acid suppressants: a retrospective cohort analysis. **BMJ**. 340 (2010) c2608 doi:10.1136/bmj.c2608

185. MYLES, P.R. [et al] – The impact of statins, ACE inhibitors and gastric acid suppressants on pneumonia mortality in a UK general practice population cohort. **Pharmacoepidemiol Drug Saf.** 18;8 (2009) 697-703.

186. ESTBORN, L.; JOELSON, S. – Occurrence of community-acquired respiratory tract infection in patients receiving esomeprazole: retrospective analysis of adverse events in 31 clinical trials. **Drug Saf.** 31 (2008) 627-636.

187. HYAMS, S.W.; KEROUB, C. – Glaucoma due to diazepam. **Am J Psychiatry.** 134;7 (1977) 447-448.

188. KADOI, C. [et al] – Bilateral angle closure glaucoma and visual loss precipitated by antidepressant and antianxiety agents in a patient with depression. **Ophthalmologica.** 214;5 (2000) 360-361.

189. BIEGANOWSKI, L.; CZERWIŃSKI, M. – Decompensation of glaucoma after administration of tricyclic antidepressants and anxiolytic drugs. **Pol Tyg Lek.** 35;38 (1980) 1457-1458.

190. SOULAIRAC, A. [et al] – [Acute glaucoma episode improved by a benzodiazepine: dipotassic clorazepate]. **Ann Med Psychol (Paris).** 2;3 (1972) 400-402.

191. DEMAILLY, P.; PAYCHA, F. – [Chlorazepate and glaucomas]. **Bull Soc Ophtalmol Fr.** 78;8-9 (1978) 585-588.

192. CALIXTO, N.; ANDRÉ DE COSTA MAIA, J. – Influence of lorazepam on ocular pressure in patients with glaucoma. **Curr Ther Res Clin Exp.** 17;2 (1975) 156-160.

193. FRITZE, J.; SCHNEIDER, B.; WEBER, B. – [Benzodiazepines and benzodiazepine-like anxiolytics and hypnotics: the implausible contraindication of closed angle glaucoma]. **Nervenarzt.** 73;1 (2002) 50-53.

194. CISZKOWSKI, C. [et al] – Codeine, ultrarapid-metabolism genotype, and postoperative death. **N Engl J Med.** 361;8 (2009) 827-828.

195. KELLY, L.E. [et al] – More codeine fatalities after tonsillectomy in North American children. **Pediatrics.** 129;5 (2012) e1343-e1347.

196. GOLDMAN, J.L. [et al] – Mortality and major morbidity after tonsillectomy: etiologic factors and strategies for prevention. **Laryngoscope.** 123;10 (2013) 2544-53.

197. HPS2-THRIVE COLLABORATIVE GROUP – HPS2-THRIVE randomized placebo-controlled trial in 25 673 high-risk patients of ER niacin/laropiprant: trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. **Eur Heart J.** 34;17 (2013) 1279-1291.

CHAPTER VI – DRUG-SAFETY ALERTS ISSUED BY REGULATORY AUTHORITIES: USEFULNESS OF META-ANALYSIS IN PREDICTING EARLIER RISKS

VI. DRUG-SAFETY ALERTS ISSUED BY REGULATORY AUTHORITIES: USEFULNESS OF META-ANALYSIS IN PREDICTING EARLIER RISKS

VI.1. ABSTRACT

To evaluate how risk estimates generated from cumulative meta-analysis performs over time for drugs having their benefit/risk ratio reevaluated due to safety issues and, additionally, assess if the results are consistent with regulatory authorities' conclusions. Four major regulatory authorities were searched for their issued safety alerts which have been supported by longitudinal, comparative studies (experimentals and/or observationals). Random-effects model was used to pooled ORs over time, by including studies according to the year they first became available. Seventeen safety alerts were included in this study. In 2008, proton pump inhibitors were associated with an increased risk for bone fractures (OR 1.25, 95% CI 1.00-1.55, P=0.049); FDA issued a safety alert in 2010 and added warnings to label. An increased risk for Clostridium difficile associated diarrhea was pooled for proton pump inhibitors in 2004 (OR 1.89, 1.19-3.02, P=0.007); FDA issued a safety alert in 2012, adding warnings to label. Proton pump inhibitors were associated with pneumonia in 2009 (OR 1.40, 1.06-1.85, P=0.017); FDA issued an alert in 2012 but concluded that B/R ratio remains positive. Statins were associated to an increased risk for diabetes (OR 1.07, 1.01-1.15, P=0.033) in 2008. EMA issued an alert in 2012, including warnings to label. The remaining cumulative meta-analyses have not estimated increased risks in advance to regulatory decisions. This study demonstrates that meta-analysis may help predicting iatrogenic risks. However, between-studies heterogeneity can considerably affect the estimated results and, therefore, this technique should not replace further assessments during benefit/risk ratio reevaluations.

VI.2. INTRODUCTION

After a medicine has been issued a market authorization and became available, unknown ADRs can arise from the everyday practice (MADRE *et al.*, 2006). This additional knowledge of the safety profile deserves to be carefully evaluated for the protection of patients (MADRE *et al.*, 2006). Some ADRs are serious enough to change the benefit/risk profile of a particular drug, leading to restriction on its use or even market withdrawal (MOORE, SINGH and FURBERG, 2012). In order to keep the patients and health care professionals updated, authorities frequently issue drug safety alerts informing about benefit/risk ratio reevaluations being conducted and subsequent regulatory decisions (US FOOD AND DRUG ADMINISTRATION, 2013; EUROPEAN MEDICINES AGENCY, 2013).

Clinical trials provide the best design to evaluate the efficacy of drug and its most common adverse effects (WYSOWSKI and SWARTZ, 2005; MEDICINES AND HEALTHCARE PRODUCTS REGULATORY AGENCY, 2011). However, not all harmfull effects can be easily anticipated in RCTs, and even if measured their reporting is usually inadequate. Observational studies usually support regulatory decisions on rare and/or long latency AEs, such as fractures, cardiovascular events or malignancies (VAN STAA, LEUFKENS and COOPER, 2001).

Different types of epidemiological data support pharmacovigilance activities, and its collection and evaluation are crucial steps for regulatory authorities in order to establish the most accurate benefit/risk ratio (PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006; US FOOD AND DRUG ADMINISTRATION, 2012). Post-marketing drug safety surveillance can be considered a dynamic prospective process that requires timelier ascertainment of drug risks together with higher quality and better documented scientific evidence (MADRE *et al.*, 2006). Therefore, a considerable period of time can separate the arising of evidence supporting the association between a new AE and a drug exposure, leading to a decision from regulatory authorities.

Meta-analysis provides the conceptual and quantitative framework for rigorous literature review, combining effect measures when appropriate and allowing an objective presentation and analysis of the available data (KIM and BERLIN, 2006). This technique has been commonly used to pool data from RCTs mainly to evaluate efficacy endpoints (ALVES, BATEL-MARQUES and MACEDO, 2012a) Despite not frequently used to evaluate safety issues, the meta-analytic cumulative analysis of evidence has demonstrated that appropriate and

timely decisions could have been taken concerning cardiovascular events associated with rofecoxib (JÜNI *et al.*, 2004).

This study is aimed to evaluate how risk estimates generated from cumulative meta-analysis performs over time for drugs having their benefit/risk ratio reevaluated due to safety issues and, additionally, assess if the results are consistent with regulatory authorities' conclusions.

VI.3. METHODS

VI.3.1. SAFETY ALERTS SELECTION

A previous study reviewed the type and publication status of data sources supporting benefit/risk ratio reevaluations conducted by FDA, Health Canada, EMA and Australian TGA (ALVES, MACEDO and BATEL MARQUES, 2013). A total of 59 safety alerts were evaluated. Only safety alerts regarding the evaluation of a causal relation between a suspected drug exposure and the occurrence of an AE which have been issued for the first time between January 2010 and December 2012 were considered for inclusion. Natural and healthcare products, medical devices, contrast agents, drug-drug interactions, drug-food interactions, medication errors, evaluations of lack of efficacy and AEs occurring during off-label use were not considered for inclusion. Only safety alerts concerning drugs with market authorization and simultaneously included in one of the thirty most prescribed drug classes worldwide used in the ambulatory setting were considered for inclusion. The complete methodology by which the safety alerts have been selected is described in the previous study (ALVES, MACEDO and BATEL MARQUES, 2013).

This study only included safety alerts in which regulatory authorities' decisions were supported by longitudinal, comparative studies [RCTs and/or observational studies (cohort or case-control)]. Studies included in meta-analyses used to support regulatory decisions were retrieved and pooled in the respective cumulative meta-analyses. No further bibliographic references were requested to regulatory authorities beyond those published in the websites. Bibliographic electronic searches were not conducted.

The following information from each safety alert was extracted: (1) date of first publication; (2) regulatory authority issuing the safety alert; (3) suspected drug(s); (4) AE; (5) type of studies supporting the evaluation; (6) drug label' section(s) updated. "Benefit/risk ratio unchanged" was considered when no change was performed.

Updates of the same safety alert were reviewed in order to retrieve further information. Two safety alerts were considered to be evaluating the same clinical question when they assessed the same AE for the same suspected drug(s). Clinical question is referring to the investigational hypothesis evaluated by a regulatory authority. Regulatory authorities could have decided to remain the suspected drug(s) under revision despite labelling changes have been carried out.

VI.3.2. META-ANALYSIS

For each clinical question, a cumulative meta-analysis was performed for the outcome of interest to display the pooled evidence over time. In the cumulative meta-analysis the studies were included according to the year they first became available – i.e., the earliest of: online publication date (Epub ahead of print date) or the correspondent journal issue publication date. Studies must have provided risk estimates (RR, OR, or HR) for patients treated with the suspected drug compared with a control group, or data allowing calculation of such risk estimates. The most adjusted estimate was used for studies presenting more than one risk estimate. A minimum of three studies was needed in order to carry on a cumulative meta-analysis.

Meta-analyses were conducted using the DerSimonian and Laird random-effects model in order to pool the OR with their 95% CIs (DERSIMONIAN and LAIRD, 1986). It was assumed that OR was an unbiased estimate of the RR. This model was chosen since it is more conservative than a fixed-effect model in the presence of between-studies heterogeneity. Between-studies heterogeneity was assessed by calculating a chi-square test and the I^2 measure of inconsistency (HIGGINS *et al.*, 2003). The influence of studies' publication date over the primary outcomes' risk considered in each safety alert was assessed by means of a meta-regression, according to the method of moments. The publication bias was visually examined by a funnel plot and statistically evaluated by Egger's regression asymmetry test (BORENSTEIN *et al.*, 2009; EGGER *et al.*, 1997). A sensitivity analysis was performed to explore the influence of studies' design in the summary estimates.

All statistical analyses were performed using the Comprehensive Meta-analysis Version 2 (Biostat, Englewood, NJ, USA).

VI.4. RESULTS

Figure VI.1 summarizes the selection process of the safety alerts. Of the 59 safety alerts, 39 were excluded since they were not supported by longitudinal, comparative studies. Twenty safety alerts were selected for further revision, of which three were excluded: valproate and impaired cognitive development since the revised studies did not provide data to calculate RR estimates; lamotrigine and increased risk of sudden unexpected death since any death occurred in studies where patients were treated with lamotrigine; and antiepileptics and bone disorders since a considerable proportion of studies compared patients with epilepsy receiving treatment with healthy individuals.

The characteristics of the safety alerts included are described in Table VI.1. The 17 safety alerts evaluated 9 different clinical questions. Two clinical questions (statins and increased blood sugar; statins and cognitive side effects) were evaluated by FDA in the same safety alert released on 28-02-2012. Four clinical questions were evaluated by only one regulatory authority. Five clinical questions were evaluated by at least two regulatory authorities.

Table VI.2 describes the results of cumulative meta-analyses over time according to the year of publication of each study, the meta-regression estimates and publication bias assessment.

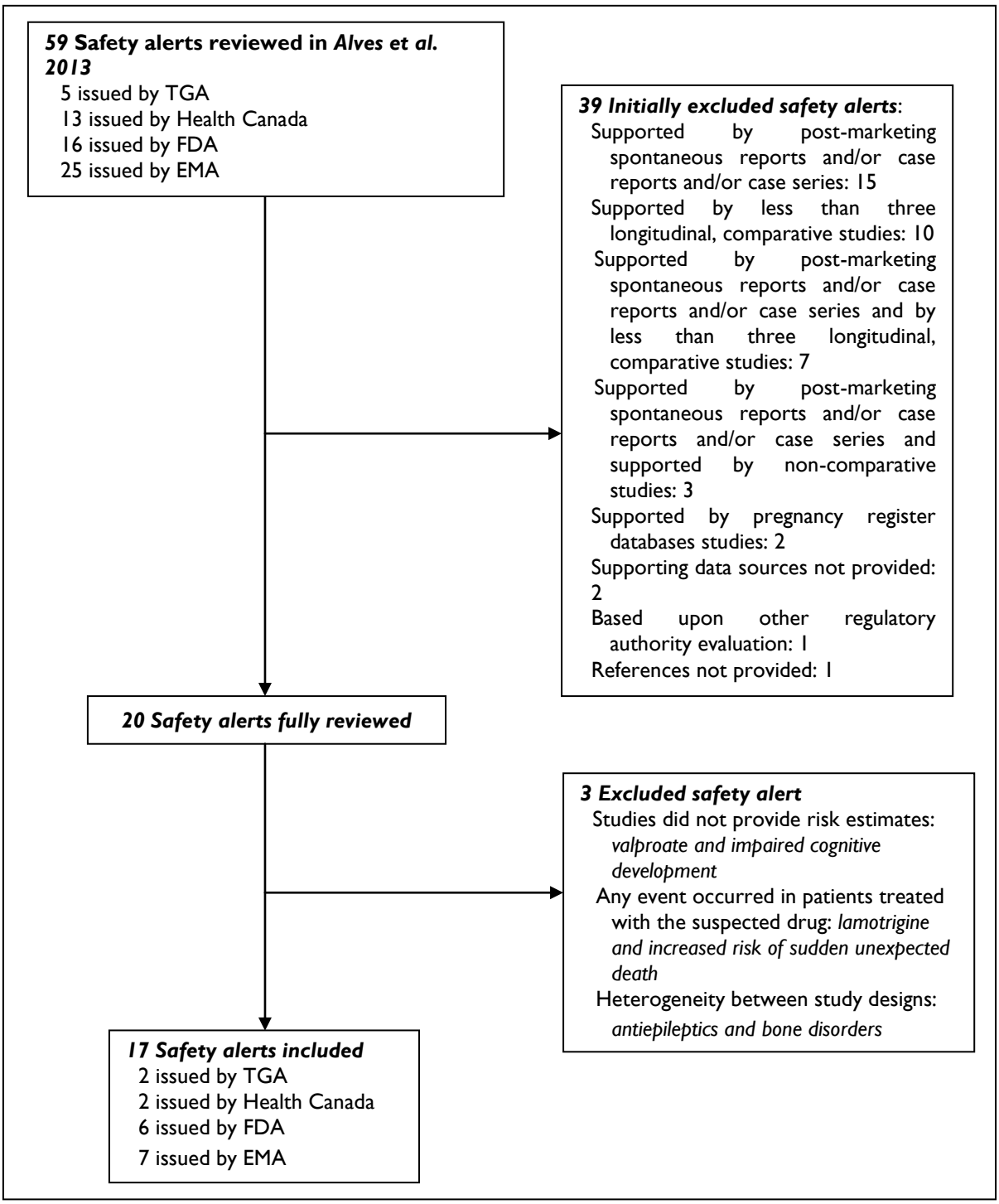


Figure VI.1 - Flow diagram of identification of safety alerts selected for cumulative meta-analysis

Table VI.1 - Safety alerts, data sources evaluated, studies included in cumulative meta-analyses and decisions taken by the regulatory authorities.

Date	Regulatory authority	Evidence supporting regulatory decision	Studies included in the cumulative meta-analysis	Label Sections update/regulatory decision
Fluoxetine and cardiovascular birth defects				
25-02-2010	EMA	1 cohort, 1 meta-analysis (9 observational studies)[1,2]	4 case-control, 2 cohort, 1 retrosp. cohort [1,3-8]	Pregnancy section updated
Proton pump inhibitors and bone fractures				
25-05-2010	FDA	5 case-control, 2 cohort, 1 cross-sectional[9-16]	6 case-control, 4 cohort, 1 retrosp. cohort[9-15,17,18,21,22]	Warnings and precautions; dosage recommendations
22-03-2012	EMA	5 case-control, 3 cohort, 2 meta-analysis (11 observational studies), 1 retrosp. cohort[9-15,17-20]		Warnings and precautions
Angiotensin receptor blockers and cancer				
15-07-2010	FDA	3 Meta-analysis of RCTs, 1 cohort[23-26]	23 RCTs, 1 cohort[26-50]	Benefit/risk ratio remains positive
20-10-2011	EMA	1 Meta-analysis of RCTs[23] *RCTs, observational studies (unspecified)* [a]		Benefit/risk ratio remains positive
Pioglitazone and bladder cancer				
17-09-2010	FDA	1 RCT, 1 cohort[51,52]	2 cohort, 1 case-control, 1 RCT[51-54]	Warnings and precautions
16-03-2011	EMA	*Post-marketing spontaneous reports* 2 cohort, 1 RCT, 1 case-control, 1 meta-analysis of RCTs[51-54,b]		Warnings and precautions; contraindications
17-06-2011	Health Canada	*Post-marketing spontaneous reports* 1 RCT, 1 cohort[51,52]		Warnings and precautions; contraindications
18-07-2011	TGA	2 cohort[52,53]		Precautions
Combined hormonal contraceptives containing drospirenone and venous thromboembolism				
27-05-2011	EMA	4 case-control, 2 cohort, 1 retrosp. cohort[55-61]	4 case-control, 2 cohort, 1 retrosp. cohort[55-61]	Warnings and precautions; contraindications
31-05-2011	FDA	3 case-control, 2 cohort, 2 retrosp. cohort[55-60, c]		Warnings and precautions; *remains under revision*
07-06-2011	Health Canada	2 case-control[55,56], *Other studies (unspecified)* [a]		Warnings and precautions; contraindications
06-07-2011	TGA	2 case-control[55,56], *Other studies (unspecified)* [a]		Precautions; contraindications; *remains under revision*
Statins and increased blood sugar				
10-01-2012	EMA	3 RCTs, 1 meta-analysis (13RCTs)[62-65]	16 RCTs, 1 cohort[62,63,67,73-87]	Warnings and precautions
28-02-2012*	FDA	*Review*[66] 3 RCTs, 3 meta-analysis (16 RCTs), 1 cohort, 1 retrosp. cohort[62,67-73]		Warnings and precautions
Proton pump inhibitors and Clostridium difficile associated diarrhea				
08-02-2012	FDA	*Post-marketing spontaneous reports, Case-reports* [a] 17 case-control, 5 retrosp. cohort, 3 cohort, 2 meta-analyses (30 observational studies)[88-113]	24 case-control, 5 retrosp. cohort, 3 cohort[88-100,102-104,106-120]	Warnings and precautions; *remains under revision*
Statins and cognitive side effects				
28-02-2012*	FDA	*Post-marketing spontaneous reports* *2 case report, 1 revision of database with post-marketing spontaneous reports, 1 survey (prospective)*[121-124] 4 RCTs, 2 cohort, 1 case-control, 1 cross-sectional, 1 meta-analysis (7 observational studies), 1 survey (retrospective)[125-134]	6 cohort, 2 case-control[132-139]	Adverse drug reactions section updated
Proton pump inhibitors and pneumonia				
26-07-2012	EMA	*Post-marketing spontaneous reports* *Systematic review*[140]*, randomized clinical trial*[a] 10 case-control, 5 retrosp. cohort, 4 cohort, 3 meta-analyses (12 observational studies), 1 pooled-analysis of RCTs, 1 retrosp., non-comparative study[141-164]	10 case-control, 5 retrosp. cohort, 4 cohort[146-164]	Benefit/risk ratio remains positive; remains under review

Notes: * - issued in the same safety alert; a – reference not provided; b – meta-analysis of RCTs unpublished; c – retrospective cohort unpublished; 24 – published at 29-11-2010; 25 – published at April 2011; w26 – published at 11-04-2011; 52 – published at 21-03-2011; 53 – published at 31-03-2012; 54 – published at 31-05-2012; 89 – study designed as being simultaneously case-control and cohort; 67, 101 – published as short communication; The references list is presented at Supplemental Data VI.1.

Fluoxetine and cardiovascular birth defects

Fluoxetine was not associated with a significant risk for cardiovascular birth defects development (final result OR 1.19; 95% CI 0.86-1.65, P=0.304; I^2=28.3%, P=0.21). Only two studies reported an increased risk (Supplemental Data VI.1 References 1,4).

Protons pump inhibitors and bone fractures

A statistically significant increased risk for bone fractures associated with proton pump inhibitors was initially identified in 2006 by pooling data from 2 studies (OR 1.31, 95% CI 1.08-1.59, P=0.007). In 2008, the risk became not statistically significant (OR 1.18, 95% CI 0.93-1.48, P=0.169) with the publication of Kaye et al. (2008) (Supplemental Data VI.1 Reference 15). In the same year, a statistically significant association could be pooled from studies after the publication of Targownik et al. (2008) (Supplemental Data VI.1 Reference 16) (OR 1.25, 955 CI 1.00-1.55, P=0.049) as well as to the final result (OR 1.27, 95% CI 1.17-1.37, P<0.001; I^2=77.0%, P<0.001).

Angiotensin receptor blockers and cancer

Angiotensin receptor blockers are not associated with an increased risk for cancer (final result OR 0.99, 95% CI 0.95-1.05, P=0.674; I^2=0%, P=0.49). Meta-regression showed that the results were stable over time [Estimate (SE) 0.001 (0.006); P=0.85].

Pioglitazone and bladder cancer

A statistically significant risk for bladder cancer associated with pioglitazone was identified after the publication of the first study on 2012 (OR 1.24, 95% CI 1.04-1.48, P=0.012) and remained significant when the results of all studies were pooled (OR 1.32, 95% CI 1.08-1.62, P=0.020; I^2=37.4%, P=0.19).

Combined hormonal contraceptives containing drospirenone and venous thromboembolism

Two early studies published in 2007 didn't report an increase in the risk between combined hormonal contraceptives containing drospirenone venous thromboembolism. Later studies established an increased risk which is confirmed in 2011 by meta-analysis [OR 1.70, 95% CI 1.13-2.57, P=0.011; I^2=81.0%, P<0.001; meta-regression estimate (SE) = 0.22 (0.12); P=0.06].

Statins and increased blood sugar

The outcome of interest evaluated was "newly-diagnosed diabetes mellitus". The cumulative meta-analysis of studies in 2008 associated statins with an increased risk for diabetes mellitus (OR 1.07, 95% CI 1.01-1.15, P=0.034). The result became statistically non-significant after data from a cohort study being pooled (OR 1.11, 95% CI 0.99-1.23, P=0.055; I^2=72.7%, P<0.001) [meta-regression estimate (SE) = 0.02 (0.006); P<0.001] (Supplemental Data VI.1 Reference 73).

Protons pump inhibitors and Clostridium difficile associated diarrhea

Cumulative meta-analysis showed that statistically significant increased risk for Clostridium difficile associated diarrhea with proton pump inhibitors became evident when the fifth study was published in 2005 (OR 1.89, 95% CI 1.19-3.02, P=0.007). Final OR estimates was 1.94 (95% CI 1.61-2.37, P<0.001; I^2=87.9%, P<0.001).

Statins and cognitive side effects

A protective effect of statins on dementia and cognitive impairment was found (OR 0.65, 95% CI 0.43-0.98, P=0.039; I^2=75.9%, P<0.001). Meta-regression showed that the results were stable over time [Estimate (SE) -0.0008 (0.06), P=0.99].

Proton pump inhibitors and pneumonia

Cumulative meta-analysis showed that statistically significant increased risk for pneumonia associated with proton pump inhibitors became evident when the third study was published in 2007 (OR 1.56, 95% CI 1.31-1.87, P<0.001). However, when the study of Roughead et al. was published in 2009, the result became statistically non-significant (OR 1.37, 95% CI 0.99-1.89, P=0.055) (Supplemental Data VI.1 Reference 154). Following the publication of Myles et al. (2009) an increased risk was observed again (OR 1.40, 95% CI 1.06-1.85, P=0.017) (Supplemental Data VI.1 Reference 150). The final OR for cumulative meta-analysis was 1.35 (95% CI 1.13-1.61, P=0.001; I^2=95.7%, P< 0.001).

Table VI.2 - Cumulative ORs and 95% CIs.

Safety alerts	**Studies**	**Design**	**Year**	**Cumulative OR**		**Heterogeneity**		**Meta-regression**		**PB[a]**
				OR (95% CI)	*P*	I^2	*P*	*Estimate (SE)*	*P*	*P*
Fluoxetine and cardiovascular birth defects *EMA 25-02-2010*	Chambers *et al.*	Cohort	1996	4.19 (0.43-40.78)	0.217					
	Malm *et al.*	Case-control	2005	1.78 (0.78-4.05)	0.172					
	Källén *et al.*	Case-control	2007	1.27 (0.79-2.03)	0.308					
	Alwan *et al.*	Case-control	2007	1.24 (0.87-1.77)	0.231					
	Louik *et al.*	Case-control	2007	1.10 (0.83-1.46)	0.503					
	Oberlander *et al.*	R. cohort	2008	1.06 (0.82-1.39)	0.652					
	Diav-Citrin *et al.*	Cohort	2008	1.19 (0.86-1.65)	0.304	28.3%	0.21	-0.102 (0.097)	0.29	0.06
Proton pump inhibitors and bone fractures *US FDA 25-05-2010* *EMA 22-03-2012*	Vestergaard *et al.*	Case-control	2006	1.18 (1.04-1.33)	0.008					
	Yang *et al.*	**Case-control**	**2006**	**1.31 (1.08-1.59)**	**0.007**					
	Kaye et al.	*Case-control*	*2008*	*1.18 (0.93-1.48)*	*0.169*					
	Targownik *et al.*	**Cohort**	**2008**	**1.25 (1.00-1.55)**	**0.049**					
	Yu *et al.*	Cohort	2008	1.25 (1.06-1.48)	0.007					
	Roux *et al.*	Cohort	2008	1.29 (1.08-1.53)	0.004					
	de Vries *et al.*	R. cohort	2009	1.25 (1.09-1.41)	0.001					
	Gray *et al.*	Cohort	2010	1.24 (1.13-1.37)	<0.001					
	Corley *et al.*	Case-control	2010	1.25 (1.15-1.36)	<0.001					
	Pouwels *et al.*	Case-control	2010	1.24 (1.15-1.34)	<0.001					
	Chiu *et al.*	Case-control	2010	1.27 (1.17-1.37)	<0.001	77.0%	< 0.001	-0.0002 (0.027)	0.99	0.12
Angiotensin receptor blockers and cancer *US FDA 15-07-2010* *EMA 20-10-2011*	IRMA 2	RCT	2001	1.27 (0.26-6.13)	0.767					
	RENAAL	RCT	2001	1.31 (0.44-3.91)	0.627					
	IDNT	RCT	2001	0.86 (0.53-1.40)	0.556					
	Val-HeFT	RCT	2001	0.89 (0.72-1.11)	0.313					
	LIFE	RCT	2002	1.04 (0.92-1.18)	0.556					
	ALPINE	RCT	2003	1.04 (0.92-1.18)	0.557					
	CHARM Alternative	RCT	2003	1.04 (0.92-1.17)	0.578					
	VALIANT	RCT	2003	1.02 (0.91-1.14)	0.759					
	CHARM	RCT	2004	1.02 (0.93-1.13)	0.654					
	VALUE	RCT	2006	0.96 (0.88-1.03)	0.255					
	TROPHY	RCT	2006	0.95 (0.88-1.03)	0.221					
	SCOPE	RCT	2007	0.97 (0.90-1.04)	0.365					
	JIKEI	RCT	2007	0.97 (0.90-1.04)	0.366					
	ONTARGET (vs ACEi)	RCT	2008	0.99 (0.93-1.05)	0.736					
	PROFESS	RCT	2008	0.99 (0.93-1.04)	0.619					
	TRANSCEND	RCT	2008	1.00 (0.95-1.05)	0.986					
	DIRECT (Overall)	RCT	2008	1.01 (0.95-1.08)	0.726					
	I-PRESERVE	RCT	2008	1.01 (0.95-1.07)	0.828					
	GISSI-AF	RCT	2009	1.01 (0.95-1.07)	0,846					
	HIJ-CREATE	RCT	2009	1.00 (0.95-1.06)	0.900					
	KYOTO	RCT	2009	1.00 (0.95-1.06)	0.939					
	NAVIGATOR	RCT	2010	1.01 (0.96-1.06)	0.749					
	ACTIVE-I	RCT	2011	0.99 (0.95-1.05)	0.943					
	Pasternak *et al.*	Cohort	2011	0.99 (0.96-1.02)	0.674	0%	0.49	0.001 (0.006)	0.85	0.56
Pioglitazone and bladder cancer *EMA 17-09-2010* *US FDA 16-03-2011* *Health Canada 17-06-2011* *TGA 18-07-2011*	Dormandy et al.	RCT	2005	2.84 (1.02-7.89)	0.045					
	Lewis et al.	Cohort	2011	1.59 (0.72-3.52)	0.162					
	Neumann et al.	**Cohort**	**2012**	**1.24 (1.04-1.48)**	**0.012**					
	Azoulay et al.	Case-control	2012	1.32 (1.08-1.62)	0.020	37.4%	0.19	-0.09 (0.08)	0.23	0.07
Combined hormonal contraceptives containing drospirenone and venous thromboembolism *EMA 27-05-2011* *US FDA 31-05-2011* *Health Canada 07-06-2011* *TGA 06-07-2011*	Dinger et al.	Cohort	2007	0.90 (0.57-1.42)	0.652					
	Seeger et al.	R. cohort	2007	0.90 (0.63-1.29)	0.566					
	Lidegaard et al.	Cohort	2009	1.15 (0.73-1.82)	0.544					
	van Hylckama Vieg et al	Case-control	2009	1.61 (0.85-3.02)	0.143					
	Dinger et al.	Case-control	2010	1.45 (0.87-2.43)	0.158					
	Parkin et al.	Case-control	2011	1.62 (0.99-2.63)	0.053					
	Jick et al.	**Case-control**	**2011**	**1.70 (1.13-2.57)**	**0.011**	81.0%	< 0.001	0.22 (0.12)	0.06	0.78
Statins and increased blood sugar *EMA 10-01-2012* *US FDA 28-02-2012**	Pravastatin MSG	RCT	1993	3.02 (0.12-75.11)	0.500					
	4S	RCT	1994	1.04 (0,84-1.28)	0.750					
	AFCAPS/TEXCAPS	RCT	1998	1.02 (0,85-1.22)	0.834					
	GISSI PREVENZIONE	RCT	2000	0,98 (0,84-1.14)	0.817					
	WOSCOPS	RCT	2001	0.94 (0.82-1.08)	0.407					
	PROSPER	RCT	2002	1.01 (0.85-1.19)	0.914					
	ALLHAT	RCT	2002	1.04 (0.91-1.19)	0.551					
	ASCOT-LLA	RCT	2003	1.06 (0.95-1.19)	0.325					
	HPS	RCT	2003	1.08 (0.98-1.19)	0.110					

	LIPID	RCT	2003	1.06 (0.97-1.16)	0.216					
	PROVE-IT TIMI 22	RCT	2004	1.06 (0.97-1.15)	0.183					
	ATHEROMA	RCT	2005	1.06 (0.98-1.15)	0.154					
	MEGA	RCT	2006	1.07 (0.99-1.15)	0.091					
	CORONA	RCT	2007	1.07 (1.00-1.15)	0.051					
	GISSI HF	**RCT**	**2008**	**1.07 (1.01-1.15)**	0.033					
	JUPITER	RCT	2008	1.09 (1.03-1.16)	0.006					
	Culver et al.	*Cohort*	*2012*	*1.11 (0.99-1.23)*	*0.051*	71.08%	< 0.001	0.02 (0.006)	<0.001	0.003
Proton pump inhibitors and Clostridium difficile associated diarrhea *US FDA 08-02-2012*	Shah *et al.*	Case-control	2000	0.86 (0.47-1.59)	0.625					
	Yip *et al.*	Case-control	2001	1.61 (0.37-7.06)	0.530					
	Kyne *et al.*	Cohort	2002	1.67 (0.69-4.04)	0.253					
	Cunningham *et al.*	Case-control	2003	1.87 (0.97-3.60)	0.060					
	Dial *et al.*	**Cohort**	**2004**	**1.89 (1.19-3.02)**	**0.007**					
	Dial *et al.*	Case-control	2004	2.01 (1.36-2.99)	0.001					
	Al-Tureihi *et al.*	Case-control	2005	2.08 (1.45-2.99)	<0.001					
	Dial *et al.*	Case-control	2005	2.26 (1.66-3.08)	<0.001					
	Loo *et al.*	Case-control	2005	2.01 (1.37-2.95)	<0.001					
	Pepin *et al.*	R. cohort	2005	1.85 (1.23-2.78)	0.003					
	Modena *et al.*	Case-control	2005	1.95 (1.33-2.87)	0.001					
	Muto *et al.*	Case-control	2005	1.98 (1.38-2.84)	<0.001					
	Gillis *et al.*	Case-control	2006	1.92 (1.37-2.70)	<0.001					
	Kazakova *et al.*	Case-control	2006	1.98 (1.43-2.75)	<0.001					
	Lowe *et al.*	Case-control	2006	1.86 (1.33-2.60)	<0.001					
	Dial *et al.*	Case-control	2006	1.82 (1.34-2.46)	<0.001					
	Yearsley *et al.*	Case-control	2006	1.82 (1.37-2.43)	<0.001					
	Akhtar *et al.*	Case-control	2007	1.83 (1.40-2.39)	<0.001					
	Beaulieu *et al.*	R. cohort	2007	1.75 (1.35-2.27)	<0.001					
	Cadle *et al.*	Case-control	2007	1.81 (1.40-2.39)	<0.001					
	Dubberke *et al.*	R. cohort	2007	1.90 (1.46-2.49)	<0.001					
	Jayatilaka *et al.*	Case-control	2007	1.94 (1.49-2.51)	<0.001					
	Aseeri *et al.*	Case-control	2008	1.98 (1.54-2.56)	<0.001					
	Baxter *et al.*	Case-control	2008	1.92 (1.52-2.44)	<0.001					
	Dial *et al.*	Case-control	2008	1.90 (1.52-2.37)	<0.001					
	Dalton *et al.*	R. cohort	2009	1.90 (1.54-2.35)	<0.001					
	Debast *et al.*	Case-control	2009	1.89 (1.53-2.32)	<0.001					
	Turco *et al.*	Case-control	2010	1.92 (1.56-2.36)	<0.001					
	Bajaj *et al.*	Case-control	2010	1.95 (1.59-2.39)	<0.001					
	Howell *et al.*	Cohort	2010	1.94 (1.60-2.34)	<0.001					
	Kim *et al.*	Case-control	2010	1.96 (1.63-2.37)	<0.001					
	Linsky *et al.*	R. cohort	2010	1.94 (1.61-2.32)	<0.001	87.9%	< 0.001	0.02 (0.04)	0.59	0.14
Statins and cognitive side effects *US FDA 28-02-2012**	Jick *et al.*	Case-control	2000	0.29 (0.13-0.64)	0.002					
	Rodriguez *et al.*	Cohort	2002	0.41 (0.21-0.82)	0.011					
	Rockwood *et al.*	Case-control	2002	0.38 (0.23-0.63)	<0.001					
	Li *et al.*	Cohort	2004	0.52 (0.24-1.16)	0.111					
	Rea *et al.*	Cohort	2005	0.67 (0.39-1.15)	0.144					
	Zandi *et al.*	Cohort	2005	0.75 (0.47-1.19)	0.216					
	Beydoun *et al.*	Cohort	2011	0.67 (0.42-1.07)	0.091					
	Betterman *et al.*	Cohort	2012	0.65 (0.43-0.98)	0.039	75.9%	< 0.001	-0.0008 (0.06)	0.99	0.15
Proton pump inhibitors and pneumonia *EMA 26-07-2012*	Mallow *et al.*	Cohort	2004	1.00 (0.38-2.60)	0.999					
	Laheij *et al.*	Case-control	2004	1.62 (0.95-2.77)	0.076					
	Gulmez *et al.*	**Case-control**	**2007**	**1.56 (1.31-1.87)**	**<0.001**					
	Sarkar *et al.*	Case-control	2008	1.74 (1.37-2.21)	<0.001					
	Beaulieu *et al.*	R. cohort	2008	1.45 (1.08-1.95)	0.015					
	Marciniak *et al.*	Case-control	2009	1.46 (1.10-1.95)	0.009					
	Roughead et al.	*R. cohort*	*2009*	*1.37 (0.99-1.89)*	*0.055*					
	Myles *et al.*	**Case-control**	**2009**	**1.40 (1.06-1.85)**	**0.017**					
	Herzig *et al.*	Cohort	2009	1.39 (1.09-1.78)	0.008					
	Miano *et al.*	R. cohort	2009	1.43 (1.13-1.82)	0.003					
	Myles *et al.*(2)	Cohort	2009	1.36 (1.08-1.71)	0.008					
	Rodriguez *et al.*	Case-control	2009	1.34 (1.08-1.66)	0.007					
	Gau *et al.*	Case-control	2010	1.34 (1.08-1.63)	0.006					
	Eurich *et al.*	Case-control	2010	1.30 (1.07-1.59)	0.009					
	Dublin *et al.*	Case-control	2010	1.29 (1.07-1.55)	0.009					
	Redelmeier *et al.*	R. cohort	2010	1.26 (1.05-1.51)	0.015					
	Kasuya *et al.*	R. cohort	2010	1.28 (1.07-1.54)	0.008					
	Meijvis *et al.*	Case-control	2011	1.32 (1.13-1.61)	0.003					
	Laheij *et al.*	Cohort	2011	1.35 (1.13-1.61)	0.001	95.7%	< 0.001	-0.02 (0.04)	0.62	0.47

Notes: * - issued in the same safety alert;

VI.4.1. PUBLICATION BIAS

Egger's asymmetry test was not statistically significant for most of the analyses but was significant for the analysis of statins and increased blood sugar (P=0.003) (Table 2). After the exclusion of the cohort study, no statistically significant asymmetry was found (P=0.773) (Supplemental Data VI.1 Reference 73). Few studies were considered for pioglitazone and bladder cancer analysis, which may not allow firm conclusions despite the non-statistically significant Egger's asymmetry test (P=0.07). Subjective evaluation of publication bias was based on the visual inspection of funnel plot.

VI.4.2. SENSITIVITY ANALYSIS

The sensitivity analysis according to different study designs did not significantly change the results with respect to the observed between-studies heterogeneity (Supplemental Data VI.2). Regarding the sub-group analysis according to the studies design, three pooled risk estimates changed their timing. When only RCTs were considered to estimate the risk for increased blood sugar (newly-diagnosed diabetes mellitus) associated with statins, the estimate yielded a statistically significant increased OR (1.07, 95% CI 1.01-1.15, P=0.034). The same increased risk was observed in the cohort study, although the final pooled estimate of different study designs was non-significant (Supplemental Data VI.1 Reference 73). For the risk estimate considering only cohort designs, the ultimate increased risk for diarrhea due to Clostridium difficile associated with proton pump inhibitors was only observed in 2009 (OR 1.75, 1.00-3.07, P=0.05). The definitive increased risk for fractures associated with proton pump inhibitors was observed in 2010 (OR 1.23, 1.07-1.40, P=0.003) when only case-control studies were considered.

VI.5. DISCUSSION

The findings of this study show that, for the majority of case scenarios (7/9), the results yielded by meta-analysis were in line with the conclusions of the regulatory authorities. Warnings could have been added to the label of proton pump inhibitors in 2004 for Clostridium difficile associated diarrhea and in 2008 for bone fractures. The label of proton pump inhibitors was subject of first updates in 2012 and 2010 regarding those AEs, respectively. These two decisions were supported by observational data only. Increased blood sugar was associated with statins in 2008 after pooling data from RCTs. The inclusion of a cohort study in the estimate returned a final result which is statistically non-significant and associated with considerable heterogeneity. Statins' label was updated to properly advise users for the risk of diabetes.

However, caution is needed when interpreting these risk estimates since they could be biased by the inherent confounding variables of the included studies (KIM and BERLIN, 2006). According to the results of the sensitivity analysis, meta-analyses exclusively integrating data from RCTs had their results characterized by low heterogeneity. Frequent and acute AEs are regularly identified from RCTs, in particular when they are pre-established endpoints of interest. When regulatory authorities and investigators are dealing with rare AEs which may be present in RCTs, it is frequent to pool data using meta-analysis. This was the case when AEs as cancer and increased blood sugar were evaluated using meta-analytic technique (BANGALORE *et al.*, 2011; SATTAR *et al.*, 2010; SIPAHI *et al.*, 2010).

All the safety issues studied in this work were evaluated by at least one type of observational methodology when regulatory authorities reviewed their benefit/risk ratio. This could be due to the fact that most of these AEs being considered as rare and/or long-latency events, such as malignancies, cardiovascular events or diabetes, which are prone to be better evaluated in post-authorization safety studies. These studies offer the advantage of a naturalistic observation, which may better represent the incidence of iatrogenic events occurred in the clinical practice (PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006; VANDENBROUCKE and PSATY, 2008).

The final conclusion of benefit/risk reevaluations conducted by the regulatory authorities may contradict risk estimates pooled by meta-analytic technique. Additional data sources supporting a causal relation between an AE and a drug can be used to substantiate regulatory decisions. In cases like this meta-analyses of the existing evidence can return inconclusive results, as it was when the authorities decided to include warnings in the label

of GLP-1 receptor agonists due to acute pancreatitis risk (ALVES, BATEL-MARQUES and MACEDO, 2012b). In this study, an increased risk of pneumonia associated with proton pump inhibitors was estimated in 2009. This is in line with the results of previous meta-analyses which yielded increased risk estimates and were subsequently reviewed by EMA (EOM *et al.*, 2011; GIULIANIO, WILHELM and KALE-PRADHAN, 2012; JOHNSTONE, NERENBERG and LOEB, 2010). However, EMA recommended that no risk minimization activities should be taken at the moment and kept this class under review. The authority considered that evidence from observational studies of an association between proton pump inhibitors as a class and pneumonia was inconsistent and might be subject to residual confounding (EUROPEAN MEDICINES AGENCY, 2012). Methodological differences may also be responsible for delays in meta-analysis to yield a statistical significant result, as it was for venous thromboembolism associated with oral contraceptives containing drospirenone. Differences between venous thromboembolism definition, risk factor of included patients and type of contraceptives used as control group may explain why the first two studies published in 2007 reported a null association (JICK and HERNANDEZ, 2011). Latter studies took these methodological and clinical issues into consideration and reported increased risks (JICK and HERNANDEZ, 2011). This may help to explain why only in 2011 an increased risk was pooled by cumulative meta-analysis, the same year that all regulatory authorities suggested labels' updates.

According to meta-regression results, most of the risk estimates were stable over time. The exception was statins and increased blood sugar for which the risk progressively increased. This may suggest that conducting cumulative meta-analysis of evidence could have help regulatory authorities to take more timely decisions. Previous studies estimated that drug withdrawals from market occur in the first 2 years and that label changes take, on average, between 7 to 11 years (MOORE, SINGH and FURBERG, 2012; LESTER et al., 2013; LASSER et al., 2002). This study included the most consumed drugs worldwide in the ambulatory care which are approved by several years and have been used to treat millions of people. Recent approved drugs may be more likely to be linked with unexpected serious AEs leading to their more rapidly regulatory actions. In this study, none of the regulatory decisions led to drugs withdrawal. Postmarketing drug safety requires careful evaluation of the existing evidence by regulatory authorities. However, timely ascertainment of drug risks with higher quality and better documented scientific evidence seems to deserve improvement (MOORE, SINGH and FURBERG, 2012).

Taking into account the safety issues evaluated in this study and the correspondent regulatory decisions, it is not possible to draw definitive recommendations about the

requirements of conducting meta-analyses every time safety signals are issued from data of longitudinal, comparative studies. Observational studies are more susceptible to bias and confounding and integrating data from such designs in meta-analyses may return results with excessive heterogeneity, as it was observed for most of the cases evaluated. In the attempt to reduce such uncertainty, a sensitivity analyses based on study designs was conducted but the results did not differ significantly. When there is little heterogeneity among studies, one may be willing to accept meta-analytic evidence as helping to establish a benefit/risk ratio (KIM and BERLIN, 2006). In the presence of substantial heterogeneity is difficult to draw conclusions and the acceptance of the results may be slow. This might be one of the reasons why regulatory authorities can take several years to conclude on an increased risk in some cases addressed in this study.

Some limitations need to be noted. The safety issues addressed in this study were evaluated by four major regulatory authorities. Others beyond those authorities were not searched for safety alerts. This could result in the exclusion of important information. This study intended to analyze safety alerts and communications which included early notices about safety issues, continuing some of them under revision at this time and without being known the authorities final decisions. Only the bibliographic references used as sources of information by the regulatory authorities to support safety alerts were considered for this study. Systematic reviews of bibliographic evidence for each clinical question were not conducted; additionally, all data sources reviewed by regulatory authorities may not have been completely published in their websites. Therefore, some studies may be absent from the cumulative meta-analyses. Despite Egger's asymmetry test and visual inspection of funnel plots may not indicate publication bias for most of the cases, turning these assessments difficult since no specific bibliographic researches have been conducted. However, the extent to which regulatory authorities have taken into account all the published scientific evidence when a benefit/risk ratio is evaluated due to a safety issue was not subject of this study.

The role of meta-analysis in pharmacovigilance is a matter of ongoing debate, and efforts are being made to develop guidelines on the use of meta-analysis in drug safety assessments (COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES, 2013). However, there are a number of methodological considerations needed to take into account when designing and conducting meta-analyses, in particular when observational studies are included, in combination with RCTs or in exclusive (KIM and BERLIN, 2006; COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES, 2013). Assessment of medicines' benefit/risk ratio after a safety issue has been raised is a highly responsible scientific exercise

that should be supported by different sources of scientific evidence, sometimes with conflicting results. Nonetheless, the quality of the meta-analysis is of high importance when safety policy measures need to be taken (KIM and BERLIN, 2006). Although regulatory authorities and independent investigators may identify increased iatrogenic risks for some drugs previous to official risk minimization strategies be set on, uncertainties due to the presence of heterogeneity or even the inclusion of different study designs may delay the decision-making process. In conclusion, this study demonstrates that meta-analysis can be useful to assess drug-AE' causal relations and, therefore, is able to predict earlier iatrogenic risks. Although cumulative meta-analysis has been used to evaluate how risk estimates perform over time with success, as in the case of rofecoxib, the results can be affected by considerable heterogeneity (JÜNI *et al.*, 2004). Therefore, this technique does not replace further assessments during the benefit/risk ratio evaluation procedure.

VI.6. BIBLIOGRAPHY

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. **Diabetes Res Clin Pract.** 98;2 (2012b) 271-284.

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – Data sources on drug safety evaluation: a review of recent published meta-analyses. **Pharmacoepidemiol Drug Saf.** 21;1 (2012a) 21-33.

ALVES, C.; MACEDO, A.F.; BATEL MARQUES, F. – Sources of information used by regulatory agencies on the generation of drug safety alerts. **Eur J Clin Pharmacol.** 69;12 (2013) 2083-2094.

BANGALORE, S. [et al] – Antihypertensive drugs and risk of cancer: network meta-analyses and trial sequential analyses of 324,168 participants from randomized trials. **Lancet Oncol.** 12;1 (2011) 65-82.

BORENSTEIN, M. [et al] – **Introduction to meta-analysis.** Chichester, England: John Wiley & Sons, 2009. ISBN 978-0-470-05724-7.

COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES – CIOMS Working Group X on "Considerations for applying good meta-analysis practices to clinical safety data within the biopharmaceutical regulatory process". (2013) Available from: http://www.cioms.ch/index.php/2012-06-10-08-47-53/working-groups/working-group-x

DERSIMONIAN, R.; LAIRD, N. – Meta-analysis in clinical trials. **Control Clin Trials.** 7 (1986) 177-188.

EGGER, M. [et al] – Bias in meta-analysis detected by a simple, graphical test. **BMJ**. 315;7109 (1997) 629-634.

EOM, C.S. [et al] – Use of acidsuppressive drugs and risk of pneumonia: a systematic review and meta-analysis. **Can Med Assoc J.** 183;3 (2011) 310-319.

EUROPEAN MEDICINES AGENCY – PhVWP monthly report on safety concerns, guidelines and general matters July 2012 – Issue number: 1207. EMA/CHMP/PhVWP/438980/2012. Committee for Medicinal Products for Human Use (CHMP). (2012) Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2012/07/WC500130391.pdf

EUROPEAN MEDICINES AGENCY – Safety monitoring of medicines. (2013) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/special_topics/general/general_content_000456.jsp&mid=WC0b01ac05801ae8fb

GIULIANIO, C.; WILHELM, S.M.; KALE-PRADHAN, P.B. – Are proton pump inhibitors associated with the development of community-acquired pneumonia?: a meta-analysis. **Expert Rev Clin Pharmacol.** 5;3 (2012) 337-344.

HIGGINS, J.P. [et al] – Measuring inconsistency in meta-analysis. **BMJ**. 327(7414) (2003) 557-560.

JICK, S.S.; HERNANDEZ, R.K. – Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. **BMJ**. 342 (2011) d2151. doi: 10.1136/bmj.d2151.

JOHNSTONE, J.; NERENBERG, K.; LOEB, M. – Meta-analysis: proton pump inhibitor use and the risk of community-acquired pneumonia. **Aliment Pharmacol Ther.** 31;11 (2010) 1165-1177.

JÜNI, P. [et al] – Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. **Lancet.** 364;9450 (2004) 2021-2029.

KIM, C.J.; BERLIN, J.A. – The use of meta-analysis in pharmacoepidemiology. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 353-366.

LASSER, K.E. – Timing for new black box warnings and withdrawals for prescription medications. **JAMA**. 287;17 (2002) 2215-2220.

LESTER, J. [et al] – Evaluation of FDA safety-related drug label changes in 2010. **Pharmacoepidemiol Drug Saf.** 22;3 (2013) 302-305.

MADRE, L. [et al] – Views from Academia, Industry, and Regulatory Agencies. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 63-88.

MEDICINES AND HEALTHCARE PRODUCTS REGULATORY AGENCY – Drug Safety Update 2011;5(5): "Citalopram and escitalopram: QT interval prolongation—new maximum daily dose restrictions (including in elderly patients), contraindications, and warnings." (2011) Available from: http://www.mhra.gov.uk/Safetyinformation/DrugSafetyUpdate/CON137769

MOORE, T.J.; SINGH, S.; FURBERG, C.D. – The FDA and new safety warnings. **Arch Intern Med.** 172;1 (2012) 78-80.

PAPANIKOLAOU, P.N.; CHRISTIDI, G.D.; IOANNIDIS, J.P.A. – Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. **CMAJ**. 174;5 (2006) 635-641.

SATTAR, N. [et al] – Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. **Lancet.** 375;9716 (2010) 735-742.

SIPAHI, I. [et al] – Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. **Lancet Oncol.** 11;7 (2010) 627-636.

US FOOD AND DRUG ADMINISTRATION – Drug Safety Communications. (2014) Available from: http://www.fda.gov/Drugs/DrugSafety/ucm199082.htm

US FOOD AND DRUG ADMINISTRATION – Guidance drug safety information - FDA's Communication to Public. Centre for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER). (2012) Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM295217.pdf

VAN STAA, T.P.; LEUFKENS, H.G.; COOPER, C. – Use of inhaled corticosteroids and risk of fractures. **J Bone Miner Res.** 16 (2001) 581-588.

VANDENBROUCKE, J.P.; PSATY, B.C. – Benefits and risks of drug treatments: how combine the best evidence on benefits with the best data about adverse effects. **JAMA**. 300;20 (2008) 2417-2419.

WYSOWSKI, D.K.; SWARTZ, L. – Adverse drug event surveillance and drug withdrawals in the United States, 1969-2002: the importance of reported suspected reactions. **Arch Inter Med.** 165;12 (2005) 1363-1369.

VI.7 SUPPLEMENTAL DATA VI

VI.7.1 SUPPLEMENTAL DATA VI.1 - REFERENCES FROM STUDIES PRESENTED AT TABLE VI.1

1. DIAV-CITRIN, O. [et al] – Paroxetine and fluoxetine in pregnancy: a prospective, multicentre, controlled, observational study. Br J Clin Pharmacol. 66;5 (2008) 695-705.

2. ELI LILLY AND COMPANY CLINICAL TRIAL REGISTRY – Results Observational/Non-interventional studies. (2013) Available from: http://www.lillytrials.com/nonintstudies/result_files/13947.pdf

3. CHAMBERS, C.D. [et al] – Birth outcomes in pregnant women taking fluoxetine. **N Engl J Med.** 335;14 (1996) 1010-1015.

4. MALM, H.; KLAUKKA, T.; NEUVONEN, P.J. – Risks associated with selective serotonin reuptake inhibitors in pregnancy. **Obstet Gynecol.** 106;6 (2005) 1289-1296.

5. KÄLLÉN, B.A.; OTTERBLAD OLAUSSON, P. – Maternal use of selective serotonin re-uptake inhibitors in early pregnancy and infant congenital malformations. **Birth Defects Res A Clin Mol Teratol.** 79;4 (2007) 301-308.

6. ALWAN, S. [et al] – Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects. **N Engl J Med.** 356;26 (2007) 2684-2692.

7. LOUIK, C. [et al] – First-trimester use of selective serotonin-reuptake inhibitors and the risk of birth defects. **N Engl J Med.** 356;26 (2007) 2675-2683.

8. OBERLANDER, T.F. [et al] – Major congenital malformations following prenatal exposure to serotonin reuptake inhibitors and benzodiazepines using population-based health data. **Birth Defects Res B Dev Reprod Toxicol.** 83;1 (2008) 68-76.

9. VESTERGAARD, P.; REJNMARK, L.; MOSEKILDE, L. – Proton pump inhibitors, histamine H2 receptor antagonists, and other antacid medications and the risk of fracture. **Calcif Tissue Int.** 79;2 (2006) 76-83.

10. YANG, Y.X. [et al] – Long-term proton pump inhibitor therapy and risk of hip fracture. **JAMA**. 296;24 (2006) 2947-2953.

11. TARGOWNIK, L.E. [et al] – Use of proton pump inhibitors and risk of osteoporosis-related fractures. **CMAJ**. 179;4 (2008) 319-326.

12. CORLEY, D.A. [et al] – Proton pump inhibitors and histamine-2 receptor antagonists are associated with hip fractures among at-risk patients. **Gastroenterology.** 139;1 (2010) 93-101.

13. GRAY, S.L. [et al] – Proton pump inhibitor use, hip fracture, and change in bone mineral density in postmenopausal women. **Arch Intern Med.** 170;9 (2010) 765-771.

14. YU, E.W. [et al] – Acid-suppressive medications and risk of bone loss and fracture in older adults. **Calcif Tissue Int.** 83;4 (2008) 251-259.

15. KAYE, J.A.; JICK, H. – Proton pump inhibitor use and risk of hip fractures in patients without major risk factors. **Pharmacotherapy** 28;8 (2008) 951-959.

16. TARGOWNIK, L.E. [et al] – Proton-pump inhibitor use is not associated with osteoporosis or accelerated bone mineral density loss. **Gastroenterology.** 138;3 (2010) 896-904.

17. DE VRIES, F. – Fracture risk in patients receiving acid suppressant medication alone and in combination with bisphosphonates. **Osteoporos Int.** 20;12 (2009) 1989-1998.

18. ROUX, C. [et al] – Increase in vertebral fracture risk in postmenopausal women using omeprazole. **Calcif Tissue Int.** 84;1 (2009) 13-19.

19. KWOK, C.S.; YEONG, J.K.; LOKE, Y.K. – Meta-analysis: risk of fractures with acid-suppressing medication. **Bone.** 48;4 (2011) 768-776.

20. EOM, C.S. – Use of acid-suppressive drugs and risk of fracture: a meta-analysis of observational studies. **Ann Fam Med.** 9;3 (2011) 257-267.

21. POUWELS, S. [et al] – Use of proton pump inhibitors and risk of hip/femur fracture: a population-based case-control study. **Osteoporos Int.** 22;3 (2011) 903-910.

22. CHIU, H.F. [et al] – Use of proton pump inhibitors increased the risk of hip fracture: a population-based case-control study. **Pharmacoepidemiol Drug Saf.** 19;11 (2010) 1131-1136.

23. SIPAHI, I. – Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. **Lancet Oncol.** 11;7 (2010) 627-636.

24. BANGALORE, S. [et al] – Antihypertensive drugs and risk of cancer: network meta-analyses and trial sequential analyses of 324,168 participants from randomized trials. **Lancet Oncol.** 12;1 (2011) 65-82.

25. THE ARB TRIALISTS COLLABORATION – Effects of telmisartan, irbesartan, valsartan, and candesartan, and losartan on cancers in 15 trials. **J Hypertens.** 29;4 (2011) 623-635.

26. PASTERNAK, B. – Use of angiotensin receptor blockers and the risk of cancer. **Circulation.** 123;16 (2011) 1729-1736.

27. THE ONGOING TELMISARTAN ALONE AND IN COMBINATION WITH RAMIPRIL GLOBAL ENDPOINT TRIAL (ONTARGET) INVESTIGATORS – Telmisartan, ramipril, or both in patients at high risk for vascular events. **N Engl J Med.** 358 (2008) 1547-1559.

28. THE TELMISARTAN RANDOMIZED ASSESSMENT STUDY IN ACEI INTOLERANT SUBJECTS WITH CARDIOVASCULAR DISEASE (TRANSCEND) INVESTIGATORS – Effects of the angiotensin receptor blocker, telmisartan on cardiovascular events in high-risk patients intolerant to ACE-inhibitors: a randomized controlled trial. **Lancet.** 372 (2008) 1174-1183.

29. YUSUF, S. [et al] – Telmisartan to prevent recurrent stroke and cardiovascular events. **N Engl J Med.** 359 (2008) 1225-1237.

30. THE NATEGLINIDE AND VALSARTAN IN IMPAIRED GLUCOSE TOLERANCE OUTCOMES RESEARCH (NAVIGATOR) STUDY GROUP – Effect of valsartan on the incidence of diabetes and cardiovascular events. **N Engl J Med.** 362 (2010) 1477-1490.

31. JULIUS, S. [et al] – Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the Valsartan Antihypertensive Long-term Use Evaluation trial (VALUE) randomized trial. **Lancet.** 363 (2004) 2022-2031.

32. PFEFFER, M.A. [et al] – Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. **Lancet.** 362 (2003) 759-766.

33. LITHELL, H. [et al] – The Study on Cognition and Prognosis in the Elderly (SCOPE): principal results of a randomized double-blind intervention trial. **J Hypertens.** 21 (2003) 875-886.

34. PARVING, H.H. [et al] – The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. **N Engl J Med.** 345 (2001) 870-878.

35. BRENNER, B.M. [et al] – Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. **N Engl J Med.** 345 (2001) 861-69.

36. BERL, T. [et al] – Cardiovascular outcomes in the Irbesartan Diabetic Nephropathy Trial of patients with type 2 diabetes and overt nephropathy. **Ann Intern Med.** 138 (2003) 542-549.

37. DAHLÖF, B. [et al] – Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. **Lancet.** 359 (2002) 995-1003.

38. LINDHOLM, L.H. [et al] – Metabolic outcome during 1 year in newly detected hypertensives: results of the Antihypertensive Treatment and Lipid Profile in a North of Sweden Efficacy Evaluation (ALPINE study). **J Hypertens.** 21 (2003) 1563-1574.

39. GRANGER, C.B. [et al] – Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. **Lancet.** 362 (2003) 772-776.

40. PFEFFER, M.A. [et al] – Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. **N Engl J Med.** 349 (2003) 1893–1906.

41. JULIUS, S. [et al] – Feasibility of treating prehypertension with an angiotensin-receptor blocker. **N Engl J Med.** 354 (2006) 1685-1697.

42. MOCHIZUKI, S. [et al] – Valsartan in a Japanese population with hypertension and other cardiovascular disease (Jikei Heart Study): a randomised, open-label, blinded endpoint morbidity-mortality study. **Lancet.** 369 (2007) 1431-1439.

43. ZILE, M.R. [et al] – Mode of death in patients with heart failure and a preserved ejection fraction: results from the Irbesartan in Heart Failure With Preserved Ejection Fraction Study (I-Preserve) trial. **Circulation.** 121 (2010) 1393-1405.

44. DISERTORI, M. [et al] – Valsartan for prevention of recurrent atrial fibrillation. **N Engl J Med.** 360 (2009) 1606-1617.

45. KASANUKI, H. [et al] – Angiotensin II receptor blocker-based vs. non-angiotensin II receptor blocker-based therapy in patients with angiographically documented coronary artery disease and hypertension: the Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease (HIJ-CREATE). **Eur Heart J.** 30 (2009) 1203-1212.

46. SAWADA, T. [et al] – Effects of valsartan on morbidity and mortality in uncontrolled hypertensive patients with high cardiovascular risks: KYOTO HEART Study. **Eur Heart J**. 30 (2009) 2461-2469.

47. COHN, J.N.; TOGNONI, G. – A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. **N Engl J Med.** 345 (2001) 1667-1675.

48. THE ACTIVE I INVESTIGATORS – Irbesartan in patients with atrial fibrillation. **N Engl J Med.** 364;10 (2011) 928-938.

49. CHATURVEDI, N. [et al] – Effect of candesartan on prevention (DIRECT-Prevent 1) and progression (DIRECT-Protect 1) of retinopathy in type 1 diabetes: randomized, placebo-controlled trials. **Lancet.** 372 (2008) 1394-1402.

50. SJOLIE, A.K. [et al] – Effect of candesartan on progression of retinopathy in type 2 diabetes (DIRECT-Protect 2): a randomized placebo-controlled trial. **Lancet.** 372 (2008) 1385-1393.

51. DORMANDY, J.A. [et al] – Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. **Lancet.** 366;9493 (2005) 1279-1289.

52. LEWIS, J.D. [et al] – Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. **Diabetes Care.** 34;4 (2011) 916-922.

53. NEUMANN, A. [et al] – Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. **Diabetologia.** 55;7 (2012) 1953-1962.

54. AZOULAY, L. [et al] – The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. **BMJ**. 344 (2012) e3645 doi:10.1136/bmj.e3645.

55. PARKIN, L. [et al] – Risk of venous thromboembolism in users of oral contraceptives containing drospirenone or levonorgestrel: nested case-control study based on UK General Practice Research Database. **BMJ**. 342 (2011) d2139 doi:10.1136/bmj.d2139.

56. JICK, S.S.; HERNANDEZ, R.K. – Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. **BMJ**. 342 (2011) d2151 doi:10.1136/bmj.d2151.

57. LIDEGAARD, Ø. [et al] – Hormonal contraception and risk of venous thromboembolism: national follow-up study. **BMJ**. 339 (2009) b2890 doi:10.1136/bmj.b2890.

58. VAN HYLCKAMA, V. [et al] – The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. **BMJ**. 339 (2009) b2921. doi: 10.1136/bmj.b2921.

59. DINGER, J.C.; HEINEMANN, L.A.; KÜHL-HABICH, D. – The safety of a drospirenone-containing oral contraceptive: final results from the European Active Surveillance Study on oral contraceptives based on 142,475 women-years of observation. **Contraception.** 75;5 (2007) 344-354.

60. SEEGER, J.D. [et al] – Risk of thromboembolism in women taking ethinylestradiol/ drospirenone and other oral contraceptives. **Obstet Gynecol.** 110;3 (2007) 587-593.

61. DINGER, J. [et al] – Risk of venous thromboembolism and the use of dienogest- and drospirenone-containing oral contraceptives: results from a German case-control study. **J Fam Plann Reprod Health Care.** 36;3 (2010) 123-129.

62. RIDKER, P.M. [et al] – Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. **N Engl J Med.** 359;21 (2009) 2195-2207.

63. SHEPHERD, J. [et al] – Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. **Lancet.** 360;9346 (2002) 1623-1630.

64. SATTAR, N. [et al] – Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. **Lancet.** 375;9716 (2010) 735-742.

65. FREEMAN, D.J. [et al] – Pravastatin and the Development of Diabetes Mellitus: Evidence for a Protective Treatment Effect in the West of Scotland Coronary Prevention Study. **Circulation.** 103;3 (2001) 357-362.

66. KOSTAPANOS, M.S. [et al] – Do statins beneficially or adversely affect glucose homeostasis? **Curr Vasc Pharmacol.** 8;5 (2010) 612-631.

67. SABATINE, M.S. – High-dose atorvastatin associated with worse glycemic control: a PROVE-IT TIMI 22 substudy. **Circulation.** 110;suppl I (2004) S834.

68. RAJPATHAK, S.N. [et al] – Statin therapy and risk of developing type 2 diabetes: a meta-analysis. **Diabetes Care.** 32;10 (2009) 1924-1929.

69. SUKHIJA, R. [et al] – Effect of statins on fasting plasma glucose in diabetic and nondiabetic patients. **J Investig Med.** 57;3 (2009) 495-499.

70. KOH, K.K. [et al] – Atorvastatin causes insulin resistance and increases ambient glycemia in hypercholesterolemic patients. **J Am Coll Cardiol.** 55;12 (2010) 1209-1216.

71. THONGTANG, N. [et al] – Effects of maximal atorvastatin and rosuvastatin treatment on markers of glucose homeostasis and inflammation. **Am J Cardiol.** 107;3 (2011) 387-392.

72. MILLS, E.J. [et al] – Efficacy and safety of statin treatment for cardiovascular disease: a network meta-analysis of 170,255 patients from 76 randomized trials. **QJM**. 104;2 (2011) 109-124.

73. CULVER, A.L. [et al] – Statin use and risk of diabetes mellitus in postmenopausal women in the Women's Health Initiative. **Arch Intern Med.** 172;2 (2012) 144-152.

74. SEVER, P.S. [et al] – Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA): a multicenter randomized controlled trial. **Lancet.** 361 (2003) 1149-58.

75. COLLINS, R. [et al] – MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomized placebo-controlled trial. **Lancet.** 361 (2003) 2005-2016.

76. FREEMAN, D.J. [et al] – Pravastatin and the Development of Diabetes Mellitus: Evidence for a Protective Treatment Effect in the West of Scotland Coronary Prevention Study. **Circulation.** 103;3 (2001) 357-362.

77. KEECH, A. [et al] – Secondary prevention of cardiovascular events with long-term pravastatin in patients with diabetes or impaired fasting glucose – results from LIPID trial. **Diabetes Care.** 26 (2003) 2713-2721.

78. KJEKSHUS, J. [et al] – Rosuvastatin in older patients with systolic heart failure. **N Engl J Med.** 357 (2007) 2248-2261.

79. SHEPHERD, J. [et al] – Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomized controlled trial. **Lancet.** 360 (2002) 1623-1630.

80. NAKAMURA, H. [et al] – Primary prevention of cardiovascular disease with pravastatin in Japan (MEGA study): a prospective randomised controlled trial. **Lancet.** 368 (2006) 1155-1163.

81. DWONS, J.R. [et al] – Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. **JAMA**. 279 (1998) 1615-1622.

82. SCANDINAVIAN SIMVASTATIN SURVIVAL STUDY STUDY GROUP – Randomised trial of cholesterol lowering in 444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). **Lancet.** 344 (1994) 1383-1389.

83. ALLHAT OFFICERS AND COORDINATORS FOR THE ALLHAT COLLABORATIVE RESEARCH GROUP – The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial. Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). **JAMA**. 288 (2002) 2998-3007.

84. GISSI PREVENZIONE INVESTIGATORS (GRUPPO ITALIANO PER LO STUDIO DELLA SOPRAVVIVENZA NELL'INFARTO MIOCARDICO) – Results of the low-dose (20 mg) pravastatin GISSI Prevenzione trial in 4271 patients with recente myocardial infarction: do stopped trials contribute to overall knowledge? **Ital Heart J.** 1 (2000) 810-820.

85. TAVAZZI, L. [et al] – Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomized, double-blind, placebo-controlled trial. **Lancet.** 372 (2008) 1231-1239.

86. YOKOI, H. [et al] – Three-year follow-up results of angiographic intervention trial using an HMG-CoA reductase inhibitor to evaluate retardation of obstructive multiple atheroma (ATHEROMA) study. **Circ J.** 69 (2005) 875-883.

87. THE PRAVASTATIN MULTINATIONAL STUDY GROUP FOR CARDIAC RISK PATIENTS – Effects of pravastatin in patients with serum total cholesterol levels from 5.2 to 7.8 mmol/liter (200 to 300 mg/dl) plus two additional atherosclerotic risk factors. **Am J Cardiol.** 72 (1993) 1031-1037.

88. AL-TUREIHI, F.I.J. [et al] – Albumin, length of stay, and proton pump inhibitors: key factors in Clostridium difficile-associated disease in nursing home patients. **J Am Med Dir Assoc.** 6;2 (2005) 105-108.

89. CUNNINGHAM, R. [et al] – Proton pump inhibitors as a risk factor for Clostridium difficile diarrhoea. **J Hosp Infect.** 54 (2003) 243-245.

90. DIAL, S. [et al] – Risk of Clostridium difficile diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. **CMAJ**. 171;1 (2004) 33-38.

91. DIAL, S. [et al] – Use of gastric acid-suppressive agents and the risk of community-acquired Clostridium difficile-associated disease. **JAMA**. 294;23 (2005) 2989-2995.

92. MUTO, C. [et al] – A large outbreak of Clostridium difficile associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. **Infect Control Hosp Epidemiol.** 26;3 (2005) 273-280.

93. DIAL, S. – Proton pump inhibitor use and risk of community-acquired Clostridium difficile-associated disease defined by prescription for oral vancomycin therapy. **CMAJ**. 175;7 (2006) 745-748.

94. DIAL, S. [et al] – Patterns of antibiotic use and risk of hospital admission for Clostridium difficile infection among elderly people in Quebec. **CMAJ**. 179;8 (2008) 767-772.

95. AKHTAR, A.J.; SHAHEEN, M. – Increasing incidence of Clostridium difficile-associated diarrhea in African-American and Hispanic patients: association with the use of proton pump inhibitor therapy. **J Natl Med Assoc.** 99;5 (2007) 500-504.

96. ASEERI, M. [et al] – Gastric acid suppression by proton pump inhibitors as a risk factor for Clostridium difficile-associated diarrhea in hospitalized patients. **Am J Gastroenterol.** 103;9 (2008) 2308-2313.

97. CADLE, R.M. [et al] – Association of proton-pump inhibitors with outcomes in Clostridium difficile colitis. **Am J Health Syst Pharm.** 64;22 (2007) 2359-2363.

98. DALTON, B.R. [et al] – Proton pump inhibitors increase significantly the risk of Clostridium difficile infection in a low-endemicity, non-outbreak hospital setting. **Aliment Pharmacol Ther.** 29;6 (2009) 626-634.

99. DUBBERKE, E.R. [et al] – Clostridium difficile-associated disease in a setting of endemicity: identification of novel risk factors. **Clin Infect Dis.** 45;12 (2007) 1543-1549.

100. HOWELL, M.D. [et al] – Iatrogenic gastric acid suppression and the risk of nosocomial Clostridium difficile infection. **Arch Intern Med.** 170;9 (2010) 784-790.

101. JANARTHANAN, S. [et al] – A meta-analysis of 16 observational studies on proton pump inhibitor use and risk of Clostridium difficile associated diarrhea. **Am J Gastroenterol.** 105;Suppl 1 (2010) S378.

102. JAYATILAKA, S. [et al] – Clostridium difficile infection in an urban medical center: five-year analysis of infection rates among adult admissions and association with the use of proton pump inhibitors. **Ann Clin Lab Sci.** 37;3 (2007) 241-247.

103. KAZAKOVA, S.V. [et al] – A hospital outbreak of diarrhea due to an emerging epidemic strain of Clostridium difficile. **Arch Intern Med.** 166;22 (2006) 2518-2524.

104. KIM, J.W. [et al] – Proton pump inhibitors as a risk factor for recurrence of Clostridium-difficile-associated diarrhea. **World J Gastroenterol.** 16;28 (2010) 3573-3577.

105. LEONARD, J.; MARSHALL, J.K.; MOAYYEDI, P. – Systematic review of the risk of enteric infection in patients taking acid suppression. **Am J Gastroenterol.** 102;9 (2007) 2047-2056.

106. LINSKY, A. [et al] – Proton pump inhibitors and risk for recurrent Clostridium difficile infection. **Arch Intern Med.** 170;9 (2010) 772-778.

107. TURCO, R. [et al] – Proton pump inhibitors as a risk factor for paediatric Clostridium difficile infection. **Aliment Pharmacol Ther.** 31;7 (2010) 754-759.

108. YEARSLEY, K.A. [et al] – Proton pump inhibitor therapy is a risk factor for Clostridium difficile-associated diarrhoea. **Aliment Pharmacol Ther.** 24;4 (2006) 613-619.

109. PEPIN, J. [et al] – Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile-associated diarrhea: a cohort study during an epidemic in Quebec. **Clin Infect Dis.** 41;9 (2005) 1254-1260.

110. SHAH, S. [et al] – Gastric acid suppression does not promote clostridial diarrhoea in the elderly. **QJM**. 93;3 (2000) 175-181.

111. LOO, V.G. [et al] – A predominantly clonal multi-institutional outbreak of Clostridium diffcile-associated diarrhea with high morbidity and mortality. **N Engl J Med.** 353 (2005) 2442-2449.

112. BEAULIEU, M. [et al] – Risk of Clostridium difficile associated disease among patients receiving proton-pump inhibitors in a Quebec medical intensive care unit. **Infect Control Hosp Epidemiol.** 28;11 (2007) 1305-1307.

113. LOWE, D.O. [et al] – Proton pump inhibitors and hospitalization for Clostridium difficile-associated disease: a population-based study. **Clin Infect Dis.** 43;10 (2006) 1272-1276.

114. BAJAJ, J.S. [et al] – Clostridium difficile is associated with poor outcomes in patients with cirrhosis: a national and tertiary center perspective. **Am J Gastroenterol.** 105 (2010) 106-113.

115. DEBAST, S.B. [et al] – Clostridium difficile PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. **Environ Microbiol.** 11 (2009) 505-511.

116. BAXTER, R.; RAY, G.T.; FIREMAN, B.H. – Case-control study of antibiotic use and subsequent Clostridium difficile-associated diarrhea in hospitalized patients. **Infect Control Hosp Epidemiol.** 29 (2008) 44-50.

117. GILLIS, C.; FARDY, J.; HUTCHINSON, J. – Proton pump inhibitors: Are they a risk factor for Clostridium difficile diarrhea: A case-control study. **Gastroenterol.** 20;Suppl A (2006) 1091.

118. LOO, V.G. [et al] – A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. **N Engl J Med.** 353 (2005) 2442-2449.

119. MODENA, S. [et al] – Clostridium difficile among hospitalized patients receiving antibiotics: A case-control study. **Infect Control Hosp Epidemiol.** 26 (2005) 685-690.

120. KYNE, L. [et al] – Underlying disease severity as a major risk factor for nosocomial Clostridium difficile diarrhea. **Infect Control Hosp Epidemiol.** 23 (2002) 653-659.

121. ORSI, A.; SHERMAN, O.; WOLDESELASSIE, Z. – Simvastatin-associated memory loss. **Pharmacotherapy.** 21;6 (2001) 767-769.

122. PARKER, B.A. [et al] – Changes in memory function and neuronal activation associated with atorvastatin therapy. **Pharmacotherapy**. 30;6 (2010) 236e-240e.

123. WAGSTAFF, L.R. [et al] – Statin-associated memory loss: analysis of 60 case reports and review of the literature. **Pharmacotherapy.** 23;7 (2003) 871-880.

124. EVANS, M.A.; GOLOMB, B.A. – Statin-associated adverse cognitive effects: survey results from 171 patients. **Pharmacotherapy.** 29;7 (2009) 800-811.

125. MULDOON, M.F. [et al] – Effects of lovastatin on cognitive function and psychological well-being. **Am J Med.** 108;7 (2000) 538-546.

126. MULDOON, M.F. [et al] – Randomized trial of the effects of simvastatin on cognitive functioning in hypercholesterolemic adults. **Am J Med.** 117;11 (2004) 823-829.

127. TROMPET, S. [et al] – Pravastatin and cognitive function in the elderly. Results of the PROSPER study. **J Neurol.** 257;1 (2010) 85-90.

128. FELDMAN, H.H. [et al] – Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. **Neurology.** 74;12 (2010) 956-964.

129. ZHOU, B.; TERAMUKAI, S.; FUKUSHIMA, M. – Prevention and treatment of dementia or Alzheimer's disease by statins: a meta-analysis. **Dement Geriatr Cogn Disord.** 23;3 (2007) 194-201.

130. BENITO-LEÓN, J. [et al] – Statins and cognitive functioning in the elderly: a population-based study. **J Alzheimers Dis.** 21;1 (2010) 95-102.

131. ZAMRINI, E.; MCGWIN, G.; ROSEMAN, J.M. – Association between statin use and Alzheimer's disease. **Neuroepidemiology.** 23;1-2 (2004) 94-98.

132. BETTERMANN, K. [et al] – Statins, risk of dementia, and cognitive function: secondary analysis of the ginkgo evaluation of memory study. **J Stroke Cerebrovasc Dis.** 21;6 (2012) 436-444.

133. BEYDOUN, M.A. [et al] – Statins and serum cholesterol's associations with incident dementia and mild cognitive impairment. **J Epidemiol Community Health.** 65;11 (2011) 949-957.

134. ZANDI, P.P. [et al] – Do statins reduce risk of incident dementia and Alzheimer disease? The Cache County Study. **Arch Gen Psychiatry.** 62;2 (2005) 217-224.

135. REA, T.D. [et al] – Statin use and the risk of incident dementia: the Cardiovascular Health Study. **Arch Neurol.** 62 (2005) 1047-1051.

136. LI, G. [et al] – Statin therapy and risk of dementia in the elderly: a community-based prospective cohort study. **Neurology.** 63 (2004) 1624-1628.

137. ROCKWOOD, K. [et al] – Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. **Arch Neurol.** 59 (2002) 223-227.

138. RODRIGUEZ, E.G. [et al] – Use of lipid-lowering drugs in older adults with and without dementia: a community-based epidemiological study. **J Am Geriatr Soc.** 50 (2002) 1852-1856.

139. JICK, H. [et al] – Statins and the risk of dementia. **Lancet.** 356 (2000) 1627-1631.

140. VAN DER MAAREL-WIERINK, C.D. [et al] – Risk factors for aspiration pneumonia in frail older people: a systematic literature review. **J Am Med Dir Assoc.** 12;5 (2001) 344-354.

141. CANANI, R.B. [et al] – Therapy with gastric acidity inhibitors increases the risk of acute gastroenteritis and community acquired pneumonia in children. **Pediatrics.** 117;5 (2006) e817-e820.

142. EOM, C.S. [et al] – Use of acidsuppressive drugs and risk of pneumonia: a systematic review and meta-analysis. **Can Med Assoc J.** 183;3 (2011) 310-319.

143. GIULIANIO, C.; WILHELM, S.M.; KALE-PRADHAN, P.B. – Are proton pump inhibitors associated with the development of community-acquired pneumonia?: a meta-analysis. **Expert Rev Clin Pharmacol.** 5;3 (2012) 337-344.

144. JOHNSTONE, J.; NERENBERG, K.; LOEB, M. – Meta-analysis: proton pump inhibitor use and the risk of community-acquired pneumonia. **Aliment Pharmacol Ther.** 31;11 (2010) 1165-1177.

145. ESTBORN, L.; JOELSON, S. – Occurrence of community-acquired respiratory tract infection in patients receiving esomeprazole: retrospective analysis of adverse events in 31 clinical trials. **Drug Saf.** 31 (2008) 627-636.

146. GULMEZ, S.E. [et al] – Use of proton pump inhibitors and the risk of community-acquired pneumonia: a population-based case-control study. **Arch Intern Med.** 167;9 (2007) 950-955.

147. LAHEIJ, R.J. [et al] – Risk of community acquired pneumonia and use of gastric acid suppressive drugs. **J Am Med Assoc.** 292;16 (2004) 1955-1960.

148. MARCINIAK, C. [et al] – Examination of selected clinical factors and medication use as risk factors for pneumonia during stroke rehabilitation: a case-control study. **Am J Phys Med Rehabil.** 88;1 (2009) 30-38.

149. MEIJVIS, S.C. [et al] – Microbial evaluation of proton-pump inhibitors and the risk of pneumonia. **Eur Respir J.** 38;5 (2011) 1165-1172.

150. MYLES, P.R. [et al] – Risk of community-acquired pneumonia and the use of statins, ace inhibitors and gastric acid suppressants: a population-based case-control study. **Pharmacoepidemiol Drug Saf.** 18;4 (2009) 269-275.

151. RODRIGUEZ, L.A. [et al] – Acid suppressive drugs and community-acquired pneumonia. **Epidemiology.** 20;6 (2009) 800-806.

152. SARKAR, M.; HENNESSY, S.; YANG, Y.X. – Proton-pump inhibitor use and the risk for community-acquired pneumonia. **Ann Intern Med.** 149;6 (2008) 391-398.

153. HERZIG, S.J. [et al] – Acid-suppressive medication use and the risk for hospital-acquired pneumonia. **J Am Med Assoc.** 301;20 (2009) 2120-2128.

154. ROUGHEAD, E.E. [et al] – Proton pump inhibitors and the risk of antibiotic use and hospitalisation for pneumonia. Med J Aust. 190;3 (2009) 114-116.

155. LAHEIJ, R. [et al] – Gastric acid suppressive therapy and community-acquired pneumonia, etiology and outcome. **Gastroenterology.** 140;5,Suppl 1 (2011) S391.

156. MIANO, T.A. [et al] – Nosocomial pneumonia risk and stress ulcer prophylaxis: a comparison of pantoprazole vs ranitidine in cardiothoracic surgery patients. **Chest**. 136;2 (2009) 440-447.

157. KASUYA, Y. [et al] – Ventilator associated pneumonia in critically ill stroke patients: frequency, risk factors, and outcomes. **J Crit Care.** 26;2 (2011) 273-279.

158. DUBLIN, S. [et al] – Use of proton pump inhibitors and H2 blockers and risk of pneumonia in older adults: a population-based case-control study. **Pharmacoepidemiol Drug Saf.** 19;8 (2010) 792-802.

159. EURICH, D.T. [et al] – Recurrent community-acquired pneumonia in patients starting acid-suppressing drugs. **Am J Med.** 123;1 (2010) 47-53.

160. GAU, J.T. [et al] – Pharmacotherapy and the risk for community-acquired pneumonia. **BMC Geriatr.** 10 (2010) 45 doi:10.1186/1471-2318-10-45.

161. BEAULIEU, M. [et al] – Do proton-pump inhibitors increase the risk for nosocomial pneumonia in a medical intensive care unit?. **J Crit Care.** 23;4 (2008) 513-518.

162. MALLOW, S. [et al] – Do proton pump inhibitors increase the incidence of nosocomial pneumonia and related infectious complications when compared with histamine-2 receptor antagonists in critically ill trauma patients?. **Curr Surg.** 61;5 (2004) 452-458.

163. REDELMEIER, D.A. [et al] – Postoperative pneumonia in elderly patients receiving acid suppressants: a retrospective cohort analysis. **BMJ**. 340 (2010) c2608 doi:10.1136/bmj.c2608.

164. MYLES, P.R. [et al] – The impact of statins, ACE inhibitors and gastric acid suppressants on pneumonia mortality in a UK general practice population cohort. **Pharmacoepidemiol Drug Saf.** 18;8 (2009) 697-703.

VI.7.2. SUPPLEMENTAL DATA VI.2 – SENSITIVITY ANALYSIS - CUMULATIVE ORS AND 95% CIS ACCORDING TO DIFFERENT STUDY DESIGNS

Supplemental Table VI.1 - Cumulative ORs and 95% CIs according to different study designs.

Safety alerts	Studies	Design	Year	Cumulative OR		Heterogeneity		Meta-regression		PB[a]
				OR (95% CI)	*P*	I^2	*P*	*Estimate (SE)*	*P*	*P*
Fluoxetine and cardiovascular birth defects *EMA 25-02-2010*	MALM *et al.*	Case-control	2005	1.56 (0.64-3.78)	0.325					
	KÄLLÉN *et al.*	Case-control	2007	1.21 (0.75-1.95)	0.434					
	ALWAN *et al.*	Case-control	2007	1.21 (0.84-1.73)	0.309					
	LOUIK *et al.*	Case-control	2007	1.08 (0.81-1.43)	0.602	0%	0.702	-0.206 (0.238)	0.39	0.09
	CHAMBERS *et al.*	Cohort	1996	4.19 (0.43-40.78)	0.217					
	OBERLANDER *et al.*	R. cohort	2008	1.28 (0.29-5.75)	0.748					
	DIAV-CITRIN *et al.*	Cohort	2008	2.08 (0.54-8.01)	0.286	67.5%	0.046	-0.07 (0.152)	0.63	0.47
Proton pump inhibitors and bone fractures *US FDA 25-05-2010* *EMA 22-03-2012*	VESTERGAARD *et al.*	Case-control	2006	1.18 (1.04-1.33)	0.008					
	YANG *et al.*	**Case-control**	**2006**	**1.31 (1.08-1.59)**	**0.007**					
	KAYE et al.	*Case-control*	*2008*	*1.18 (0.93-1.48)*	*0.169*					
	CORLEY *et al.*	**Case-control**	**2010**	**1.23 (1.07-1.40)**	**0.003**					
	POUWELS *et al.*	Case-control	2010	1.23 (1.10-1.37)	<0.001					
	CHIU *et al.*	Case-control	2010	1.27 (1.12-1.43)	<0.001	79.1%	< 0.001	0.018 (0.041)	0.67	0.86
	TARGOWNIK *et al.*	Cohort	2008	1.92 (1.16-3.18)	0.011					
	YU *et al.*	**Cohort**	**2008**	**1.47 (1.03-2.08)**	**0.034**					
	ROUX *et al.*	Cohort	2008	1.66 (1.09-2.53)	0.019					
	DE VRIES *et al.*	R. cohort	2009	1.33 (1.09-1.62)	0.006					
	GRAY *et al.*	Cohort	2010	1.25 (1.13-1.39)	<0.001	65.6%	0.02	-0.083 (0.089)	0.35	0.01
Angiotensin receptor blockers and cancer *US FDA 15-07-2010* *EMA 20-10-2011*	IRMA 2	RCT	2001	1.27 (0.26-6.13)	0.767					
	RENAAL	RCT	2001	1.31 (0.44-3.91)	0.627					
	IDNT	RCT	2001	0.86 (0.53-1.40)	0.556					
	Val-HeFT	RCT	2001	0.89 (0.72-1.11)	0.313					
	LIFE	RCT	2002	1.04 (0.92-1.18)	0.556					
	ALPINE	RCT	2003	1.04 (0.92-1.18)	0.557					
	CHARM Alternative	RCT	2003	1.04 (0.92-1.17)	0.578					
	VALIANT	RCT	2003	1.02 (0.91-1.14)	0.759					
	CHARM	RCT	2004	1.02 (0.93-1.13)	0.654					
	VALUE	RCT	2006	0.96 (0.88-1.03)	0.255					
	TROPHY	RCT	2006	0.95 (0.88-1.03)	0.221					
	SCOPE	RCT	2007	0.97 (0.90-1.04)	0.365					
	JIKEA	RCT	2008	0.97 (0.90-1.04)	0.366					
	ONTARGET (vs ACEi)	RCT	2008	0.99 (0.93-1.05)	0.736					
	PROFESS	RCT	2008	0.99 (0.93-1.04)	0.619					
	TRANSCEND	RCT	2008	1.00 (0.95-1.05)	0.986					
	DIRECT (Overall)	RCT	2008	1.01 (0.95-1.08)	0.726					
	I-PRESERVE	RCT	2008	1.01 (0.95-1.07)	0.828					
	GISSI-AF	RCT	2009	1.01 (0.95-1.07)	0,846					
	HIJ-CREATE	RCT	2009	1.00 (0.95-1.06)	0.900					
	KYOTO	RCT	2009	1.00 (0.95-1.06)	0.939					
	NAVIGATOR	RCT	2010	1.01 (0.96-1.06)	0.749					
	ACTIVE-I	RCT	2011	0.99 (0.95-1.05)	0.943	2.3%	0.43	0.0037(0.008)	0.65	0.59
	PASTERNAK *et al.*	Cohort	2011	0.99 (0.95-1.03)	0.623	-	-	-	-	-
Pioglitazone and bladder cancer *EMA 27-05-2011* *US FDA 31-05-2011* *Health Canada 07-06-2011* *TGA 06-07-2011*	DORMANDY *et al.*	RCT	2005	2.84 (1.02-7.89)	0.045	-	-	-	-	-
	LEWIS *et al.*	Cohort	2011	1.20 (0.93-1.55)	0.162					
	NEUMANN *et al.*	**Cohort**	**2012**	**1.22 (1.05-1.42)**	**0.012**	0%	<0.001			
	AZOULAY *et al.*	Case-control	2012	1.83 (1.10-3.05)	0.020	-	-	-	-	-
Combined hormonal contraceptives containing drospirenone and venous thromboembolism *EMA 27-05-2011* *US FDA 31-05-2011* *Health Canada 07-06-2011* *TGA 06-07-2011*	DINGER *et al.*	Cohort	2007	0.90 (0.57-1.42)	0.652					
	SEEGER *et al.*	R. cohort	2007	0.90 (0.63-1.29)	0.566					
	LIDEGAARD *et al.*	Cohort	2009	1.15 (0.73-1.82)	0.544	72.1%	0.03	0.30 (0.112)	0.01	0.18
	VAN HYLCKAMA VIEG *et al*	Case-control	2009	6.30 (2.90-13.69)	<0.001					
	DINGER *et al.*	Case-control	2010	2.47 (0.41-14.91)	0.977					
	PARKIN *et al.*	Case-control	2011	2.68 (0.84-8.56)	0.098					
	JICK *et al.*	**Case-control**	**2011**	**2.50 (1.27-4.94)**	**0.008**	81.1%	0.001	-0.273 (0.534)	0.61	0.66
Statins and increased blood sugar *EMA 10-01-2012*	PRAVASTATIN MSG	RCT	1993	3.02 (0.12-75.11)	0.500					
	4S	RCT	1994	1.04 (0,84-1.28)	0.750					
	AFCAPS/TEXCAPS	RCT	1998	1.02 (0,85-1.22)	0.834					
	GISSI PREVENZIONE	RCT	2000	0,98 (0,84-1.14)	0.817					

*US FDA 28-02-2012**	WOSCOPS	RCT	2001	0.94 (0.82-1.08)	0.407					
	PROSPER	RCT	2002	1.01 (0.85-1.19)	0.914					
	ALLHAT	RCT	2002	1.04 (0.91-1.19)	0.551					
	ASCOT-LLA	RCT	2003	1.06 (0.95-1.19)	0.325					
	HPS	RCT	2003	1.08 (0.98-1.19)	0.110					
	LIPID	RCT	2003	1.06 (0.97-1.16)	0.216					
	PROVE-IT TIMI 22	RCT	2004	1.06 (0.97-1.15)	0.183					
	ATHEROMA	RCT	2005	1.06 (0.98-1.15)	0.154					
	MEGA	RCT	2006	1.07 (0.99-1.15)	0.091					
	CORONA	RCT	2007	1.07 (1.00-1.15)	0.051					
	GISSI HF	**RCT**	**2008**	**1.07 (1.01-1.15)**	**0.033**					
	JUPITER	RCT	2008	1.09 (1.03-1.16)	0.006	2.8%	0.42	0.012 (0.008)	0.14	0.77
	CULVER et al.	Cohort	2012	1.48 (1.38-1.59)	<0.001	-	-	-	-	-
Proton pump inhibitors and Clostridium difficile associated diarrhea *US FDA 08-02-2012*	SHAH *et al.*	Case-control	2000	0.86 (0.47-1.59)	0.625					
	YIP *et al.*	Case-control	2001	1.61 (0.37-7.06)	0.530					
	CUNNINGHAM *et al.*	Case-control	2003	1.83 (0.76-4.42)	0.179					
	DIAL *et al.*	**Case-control**	**2004**	**2.00 (1.06-3.79)**	**0.033**					
	AL-TUREIHI *et al.*	Case-control	2005	2.13 (1.22-3.70)	0.008					
	DIAL *et al.*	Case-control	2005	2.30 (1.52-3.46)	<0.001					
	LOO *et al.*	Case-control	2005	1.99 (1.24-3.22)	0.005					
	MODENA *et al.*	Case-control	2005	2.12 (1.38-3.27)	0.001					
	MUTO *et al.*	Case-control	2005	2.14 (1.46-3.15)	<0.001					
	GILLIS *et al.*	Case-control	2006	2.04 (1.42-2.93)	<0.001					
	KAZAKOVA *et al.*	Case-control	2006	2.11 (1.51-2.96)	<0.001					
	LOWE *et al.*	Case-control	2006	1.96 (1.30-2.95)	0.001					
	DIAL *et al.*	Case-control	2006	1.89 (1.32-2.71)	<0.001					
	YEARSLEY *et al.*	Case-control	2006	1.90 (1.35-2.66)	<0.001					
	AKHTAR *et al.*	Case-control	2007	1.90 (1.40-2.57)	<0.001					
	CADLE *et al.*	Case-control	2007	1.97 (1.46-2.65)	<0.001					
	JAYATILAKA *et al.*	Case-control	2007	2.01 (1.51-2.67)	<0.001					
	ASEERI *et al.*	Case-control	2008	2.07 (1.56-2.73)	<0.001					
	BAXTER *et al.*	Case-control	2008	1.98 (1.54-2.55)	<0.001					
	DIAL *et al.*	Case-control	2008	1.94 (1.54-2.45)	<0.001					
	DEBAST *et al.*	Case-control	2009	1.92 (1.53-2.41)	<0.001					
	TURCO *et al.*	Case-control	2010	1.96 (1.57-2.46)	<0.001					
	BAJAJ *et al.*	Case-control	2010	2.00 (1.61-2.50)	<0.001					
	KIM *et al.*	Case-control	2010	2.04 (1.65-2.53)	<0.001	86.9%	<0.001	0.045 (0.049)	0.37	0.10
	KYNE *et al.*	Cohort	2002	2.19 (0.89-5.41)	0.089					
	DIAL *et al.*	**Cohort**	**2004**	**2.13 (1.34-3.66)**	**0.001**					
	PEPIN et al.	*R. cohort*	*2005*	*1.54 (0.85-2.81)*	*0.164*					
	BEAULIEU *et al.*	R. cohort	2007	1.28 (0.85-1.92)	0.234					
	DUBBERKE *et al.*	R. cohort	2007	1.72 (0.85-3.47)	0.133					
	DALTON *et al.*	**R. cohort**	**2009**	**1.75 (1.00-3.07)**	**0.050**					
	HOWELL *et al.*	Cohort	2010	1.75 (1.13-2.71)	0.012					
	LINSKY *et al.*	R. cohort	2010	1.70 (1.17-2.46)	0.005	91.2%	< 0.001	-0.009 (0.08)	0.91	0.98
Statins and cognitive side effects	JICK *et al.*	Case-control	2000	0.29 (0.13-0.64)	0.002					
	ROCKWOOD *et al.*	Case-control	2002	0.28 (0.15-0.54)	<0.001	0%	<0.001	-	-	-
*US FDA 28-02-2012**	RODRIGUEZ *et al.*	Cohort	2002	0.58 (0.27-1.24)	0.161					
	LI *et al.*	Cohort	2004	0.90 (0.45-1.79)	0.763					
	REA *et al.*	Cohort	2005	1.06 (0.78-1.44)	0.712					
	ZANDI *et al.*	Cohort	2005	1.10 (0.88-1.38)	0.406					
	BEYDOUN *et al.*	Cohort	2011	0.91 (0.63-1.33)	0.632					
	BETTERMAN *et al.*	Cohort	2012	0.79 (0.53-1.18)	0.257	73.6%	0.002	-0.0087 (0.04)	0.03	0.53
Proton pump inhibitors and pneumonia *EMA 26-07-2012*	LAHEIJ *et al.*	Case-control	2004	1.89 (1.36-2.62)	<0.001					
	GULMEZ *et al.*	**Case-control**	**2007**	**1.60 (1.31-1.96)**	**<0.001**					
	SARKAR *et al.*	Case-control	2008	1.80 (1.41-2.29)	<0.001					
	MARCINIAK *et al.*	Case-control	2009	1.80 (1.43-2.26)	<0.001					
	MYLES *et al.*	Case-control	2009	1.73 (1.42-2.10)	<0.001					
	RODRIGUEZ *et al.*	Case-control	2009	1.60 (1.25-2.04)	<0.001					
	GAU *et al.*	Case-control	2010	1.54 (1.22-1.94)	<0.001					
	EURICH *et al.*	Case-control	2010	1.48 (1.18-1.85)	0.001					
	DUBLIN *et al.*	Case-control	2010	1.43 (1.15-1.78)	0.001					
	MEIJVIS *et al.*	Case-control	2011	1.48 (1.20-1.82)	0.003	92.4%	<0.001	-0.059 (0.055)	0.29	0.11
	MALLOW *et al.*	Cohort	2004	1.00 (0.38-2.60)	0.999					
	BEAULIEU *et al.*	R. cohort	2008	0.69 (0.45-1.06)	0.088					
	ROUGHEAD et al.	R. cohort	2009	0.93 (0.59-1.46)	0.742					
	HERZIG *et al.*	Cohort	2009	1.14 (0.96-1.34)	0.128					
	MIANO *et al.*	R. cohort	2009	1.10 (0.92-1.30)	0.294					
	MYLES *et al.*(2)	**Cohort**	**2009**	**1.36 (1.08-1.71)**	**0.008**					
	REDELMEIER et al.	*R. cohort*	*2010*	*1.07 (0.92-1.23)*	*0.382*					
	KASUYA *et al.*	R. cohort	2010	1.09 (0.94-1.26)	0.264					
	LAHEIJ *et al.*	Cohort	2011	1.32 (0.97-1.32)	0.107	79.2%	< 0.001	-0.116 (0.078)	0.14	0.74

CHAPTER VII – GENERAL DISCUSSION

VII. GENERAL DISCUSSION

VII.1. DISCUSSION

Despite each experimental chapter has its own discussion, this section is intended to discuss in a more integrated and broader manner all the research studies presented in this thesis. The work conducted under the presented thesis aims at answer to the general objectives proposed at the beginning of this dissertation.

The knowledge about the risk profile of a medical intervention is as important as its benefits. Both are crucial to establish the most accurate medicine's benefit-risk profile. The majority of newly introduced medical interventions have small, incremental benefits when compared with available treatments (IOANNIDIS, 2009). Therefore, differences in safety profiles should have a key role in the treatments choice (IOANNIDIS, 2009).

Regarding a pharmacologic intervention, most of the information on its safety profile is produced during the pre-market clinical development. However, rare and serious AEs are usually identified through observational pharmacovigilance activities after a drug being introduced into the market (WYSOWSKI and SWARTZ, 2005; LASSER *et al.*, 2002). Since observational studies are based on real world clinical data, they may better reflect the frequency of AEs (VANDENBROUCKE, 2004; VANDENBROUCKE, 2006). Systematically reviewing of both experimental and observational data on safety may provide a more balanced and realistic account of the likeliwood of the outcomes (HOPEWELL, WOLFENDEN, and CLARKE, 2008).

Investigate uncommon or long-term AEs associated with pharmacological treatments is an important application of meta-analysis. However, combining data from different study designs through meta-analytic technique can be affected by inherent biases of the considered studies (KIM and BERLIN, 2006; BERLIN, CEPEDA and KIM, 2012). Taking into account these considerations and the objectives of this work, the first task conducted was the identification and the evaluation of meta-analyses from both experimental and observational studies where safety was found to be an outcome measure. The search was limited to meta-analyses published in eight medical journals from 2005 to 2010 which were selected for having the highest impact factor on the area of general/internal medicine.

According to the findings, only a limited number of meta-analyses are currently devoted to evaluate the safety of pharmacological interventions. The majority of the 438

meta-analyses identified during the 5-years studied period was designed to assess efficacy or non-pharmacological interventions, while only 60 (14%) assessed drug safety as a primary outcome. It should be noted that some meta-analyses evaluated the same clinical question during the studied period since some treatments were under evaluation by regulatory authorities (e.g., risk of cardiovascular events associated with rosiglitazone; antidepressants and risk of suicidal behaviors). This research identified meta-analyses devoted to evaluate the safety of pharmacological interventions. However, of the 60 meta-analyses evaluated, two included only observational studies and four included data from both observational and experimental studies. Moreover, of the meta-analyses which included different study designs, two compared to results pooled from RCTs with those pooled from observational studies, while in other meta-analysis observational studies were used to provide information on safety outcomes which was not reported in RCTs.

The relatively low number of meta-analyses including observational data may have been influenced by the choice of this set of journals. Journals covering the disciplines of pharmacoepidemiology and pharmacovigilance may publish relevant meta-analyses, some of those including different study designs as data sources (HENNESSY *et al.*, 2001; GAGNE, GRIESDALE and SCHNEEWEISS, 2009; LOKE, JEEVANANTHAM and SINGH, 2009; TOH and HERNÁNDEZ-DÍAZ, 2007; CHEN and ASHCROFT, 2006). Nonetheless, other reasons may exist too for such few meta-analyses designed to evaluate drug safety have been find. Despite extensive study in pre-approval RCTs of safety and effectiveness, doubts can remain about their effects, whether unintended, harmful or beneficial (PLATT *et al.*, 2014). Clinical trials are not designed to provide full assessments of drugs safety profiles. Additionally, most of the investigators may fear an increase in the uncertainty due to integrate data from both experimental and observational studies in meta-analyses. Therefore, these factors may contribute to the existence of a low number of meta-analyses dedicated to evaluate safety issues.

A previous study which randomly identified 60 meta-analyses published during 1995 found that 27 of them have included observational studies. However, only 11 meta-analyses were conducted to evaluate therapeutic interventions and no distinction was made between those evaluating efficacy or safety outcomes (EGGER, SCHNEIDER and DAVEY SMITH, 1998). The type of studies included in systematic reviews has also been described. Hopewell and colleagues (2008) found that Cochrane reviews included only RCTs (95%) for both efficacy and adverse outcomes. In contrast, systematic reviews published in the Database of

Abstracts of Reviews of Effects (DARE) were more likely to include other type of studies, with 58% relying only on experimental designs (HOPEWELL, WOLFENDEN, and CLARKE, 2008).

Meta-analyses of RCTs are based on the assumption that each trial provides an unbiased estimate of the effect of an experimental treatment and that the variability of the results between the studies is attributed to random variation (EGGER, SCHNEIDER and DAVEY SMITH, 1998). Thus, the overall effect measure pooled from a sample of RCTs will provide an essentially unbiased estimate of the treatment effect (EGGER, SCHNEIDER and DAVEY SMITH, 1998; EGGER, SMITH and PHILLIPS, 2007). This assumption is strengthened when a meta-analysis includes RCTs having similar designs, follow-up time duration, end-points assessment, and patient demographics. Randomized clinical trials are designed to provide evidence on the efficacious treatment of disease and tend to be under-powered for detection of AEs. (LESKO and MITCHELL, 2012; HAUSMANN, SCHNYDER and PICHLER, 2012). Unexpected and rare AEs may not be identified in RCTs conducted during clinical development. Moreover, adverse outcomes are not always reported in a consistent way. Therefore, evidence on safety reported by RCTs and their associated meta-analyses is often insufficient.

Although potential biases and confounding have to be considered, observational studies are more likely to include a broad representation of the population at-risk and provide reliable estimates of the incidence of AEs in clinical practice. Consequently, some AEs are only identified in observational studies, years after their introduction into the (STAFFA, CHANG and GREEN, 2002; LIDEGAARD *et al.*, 2009).

In this work, the safety of the direct inhibitors of coagulation factor Xa, rivaroxaban and apixaban, and GLP-1 agonists, exenatide and liraglutide, was evaluated in two meta-analyses.

Most of the thromboprophylaxis after joint surgery consists in the use of heparins or vitamin K antagonists, such warfarin (GEERTS *et al.*, 2008). However, these therapies had specific limitations and new oral anticoagulant agents have been developed, like the direct inhibitors of coagulation factor Xa (WEITZ *et al.*, 2010; BECATTINI, LIGNANI and AGNELLI, 2010; GROSS and WEITZ, 2008). Both rivaroxaban and apixaban have shown to be effective in preventing thromboprophylaxis following knee and hip arthroplasty (LASSEN *et al.*, 2009; LASSEN *et al.*, 2010; ERIKSSON *et al.*, 2008; TURPIE *et al.*, 2009). Yet, the safety of rivaroxaban was subject of discussion in scientific literature due to doubts on the risk for haemorrhages and wound complications (JENSEN *et al.*, 2011; LOTKE, 2008; CAO *et al.*, 2010; GÓMEZ-OUTES *et al.*, 2009). Since rivaroxaban and apixaban have been developed almost at the same time, no

study directly compared both oral anticoagulants. Thus, a meta-analysis of RCTs was conducted in order to compare the safety profile of rivaroxaban and apixaban.

The results of the meta-analysis suggested that thromboprophylaxis with apixaban after knee arthroplasty was associated with a lower risk of major, clinical relevant nonmajor, and total bleeding events, when indirectly compared with rivaroxaban. When hip arthroplasty was the case, no differences were observed. A previous meta-analysis evaluating the efficacy and safety of rivaroxaban confirmed its superiority to enoxaparin as a thromboprophylaxis agent administered after joint surgery (CAO *et al.*, 2010). However, the authors did not recommended the use of rivaroxaban since a higher proportion of bleeding events was observed in patients receiving it as a treatment. The RCTs of both drugs used either the European regimen of enoxaparin, 40 mg, subcutaneously, once-daily, or the North American regimen, consisting in administer enoxaparin 30 mg, subcutaneously, twice-daily. Therefore, a sensitivity analysis was conducted in order to establish comparisons using the European enoxaparin regimen. As a result, statistical significance disappeared and both apixaban and rivaroxaban presented an identical risk for bleeding. This is suggestive of a higher risk of bleeding associated with twice-daily enoxaparin 30 mg. Although both enoxaparin regimens have never been compared, this tendency for an increased risk of bleeding with enoxaparin 30 mg twice-daily regime was reported in a previous meta-analysis (HUANG *et al.*, 2011).

The bleeding endpoints evaluated in this meta-analysis were the same as those pre-specified in the RCTs. The assessment procedure and adjudication of AEs as well as follow-up times were the same in the phase III RCTs of rivaroxaban and apixaban. Additionally, the both drugs' clinical developments programmes included patients with similar demographic characteristics. The comparable design of studies evaluating apixaban and rivaroxaban as well as the fact that only data from well-defined outcomes has been pooled may have been responsible for the lower levels of heterogeneity observed throughout this meta-analysis.

Although this meta-analysis includes results of high-quality RCTs and includes data from a sample of more than 28 000 patients, not all the studies assessed safety outcomes considered to be relevant, such as wound infection, healing or drainage rates (JENSEN *et al.*, 2011; LOTKE, 2008). An observational retrospective study compared the safety of rivaroxaban with tinzaparin in patients submitted to knee or hip arthroplasty (JENSEN *et al.*, 2011). The results have shown that patients who received treatment with rivaroxaban were more than twice as likely to return to theatre with a wound complication. The authors

stated that they discontinued the use of rivaroxaban based on their results, raising the need for further RCTs assessing the efficacy and safety of rivaroxaban in clinical practice.

Serious AEs have been linked with exenatide and liraglutide, the first two GLP-1 agonists being marketed. Since, 2005, cases of acute pancreatitis occurring in patients treated with exenatide BID have been spontaneously reported to regulatory authorities, mainly to FDA (US FOOD AND DRUG ADMINISTRATION, 2007). Analyses of spontaneous reporting systems' databases identified an increased risk for acute pancreatitis associated with exenatide BID (ELASHOFF *et al.*, 2011; RASCHI *et al.*, 2013). However, these findings were confirmed through observational longitudinal studies (DORE, SEEGER and CHAN, 2009; GARG, CHEN and PENDERGRASS, 2010; DORE *et al.*, 2011). Nonetheless, FDA decided for the update of exenatide product's labelling based on post-marketing spontaneously reported cases (US FOOD AND DRUG ADMINISTRATION, 2009). Acute pancreatitis was also identified in RCTs evaluating liraglutide (PARKS and ROSENBRAUGH, 2010).

Other serious adverse issue which has been linked with GLP-1 agonists is thyroid cancer, particularly associated with liraglutide. Benign thyroid C-cell adenomas were observed in rodents treated with exenatide BID but no carcinomas were reported (EUROPEAN MEDICINES AGENCY, 2006; DRUCKER *et al.*, 2010). In carcinogenicity studies, thyroid tumours occurred in rats administered with exenatide once-weekly (EUROPEAN MEDICINES AGENCY, 2011). During clinical development, unspecified neoplasms have been reported in patients treated with exenatide BID (EUROPEAN MEDICINES AGENCY, 2006). C-cell hyperplasia and thyroid cancer were observed in pre-clinical toxicology studies conducted for liraglutide (EUROPEAN MEDICINES AGENCY, 2009; BJERRE KNUDSEN *et al.*, 2010). Thyroid neoplasms were also reported during the liraglutide RCTs (EUROPEAN MEDICINES AGENCY, 2009; PARKS and ROSENBRAUGH, 2010). Liraglutide's product label carries a Black Box warning noticing the risk for thyroid c-cell cancer (FOOD AND DRUGS ADMINISTRATION, 2010).

In order to evaluate the risk of acute pancreatitis, any cancer or thyroid cancer, associated with GLP-1 agonists, exenatide and liraglutide, a meta-analysis was carried out based on both experimental and observational published studies. The results suggest that neither exenatide nor liraglutide increase the risk for acute pancreatitis. These findings are in line with those reported in observational studies conducted for exenatide (DORE, SEEGER and CHAN, 2009; GARG, CHEN and PENDERGRASS, 2010; DORE *et al.*, 2011). No post-marketing observational studies evaluating acute pancreatitis have been identified for liraglutide.

Although no association between GLP-1 agonists' exposure and acute pancreatitis has been established, several confounding factors should be considered. Nausea, abdominal discomfort and vomiting are ADRs known to be associated with GLP-1 agonists treatment (EUROPEAN MEDICINES AGENCY, 2006; EUROPEAN MEDICINES AGENCY, 2011; EUROPEAN MEDICINES AGENCY, 2009). These events are symptoms of acute pancreatitis, which may impair patients to recognise it and could difficult health professionals to establish a proper diagnosis (BALANI and GRENDELL, 2008). Only studies exclusively evaluating patients with type 2 diabetes mellitus have been included in this meta-analysis. There is a higher risk for developing acute pancreatitis in patients suffering from type 2 diabetes, independently of the drug therapy (NOEL *et al.*, 2009). GLP-1 agonists were initially approved as type 2 diabetes add-on therapy. Patients receiving GLP-1 agonists are more likely to be at more advanced stages of the disease and, therefore, more prone for developing acute pancreatitis. This may indicate that there is an increased risk for confounding by indication, particularly when observational studies are the case (NATHAN *et al.*, 2009; GARG, CHEN and PENDERGRASS, 2010).

This meta-analysis did not identify an increased risk for any cancer associated with exenatide. The results remained unchanged when the analysis was stratified according to the therapeutic regimens or different comparators. No significant difference in the risk for cancer was observed regarding treatment with liraglutide, except when the analysis was stratified based on studies methodological quality. When only five high methodological quality studies where considered, it was observed an increased risk of cancer from all causes in patients treated with liraglutide. Nonetheless, caution should be taken when interpreting this result, since is the only significant association found, suggesting a possible chance of finding. The scale of Downs and Black (1998) was chosen since it is able to assess both experimental and observational studies. The number of quality assessment scales that exist make it unclear how to achieve the best assessment and results may vary depending on the scale used (JÜNI *et al.*, 1999).

Although no consistent increased risk was found for both drugs, a divergence between the risk of cancer associated with exenatide and liraglutide was identified (-14% for exenatide and +35% for liraglutide, both non-significant). When only high quality studies were considered, this difference increases. Only RCTs were included in the meta-analysis evaluating the risk of cancer associated with GLP-1 agonists since observational studies were not identified in our search strategy. Considering that cancer is a long-latency event, the follow-up duration of the experimental studies may not be long enough to establish a reliable causality association between liraglutide exposure and cancer occurrence. Despite the few

cases identified during RCTs, the proportion of patients diagnosed with thyroid carcinomas was higher in those receiving liraglutide when comparing with those receiving any control treatment; no such cases were observed in patients receiving exenatide. The small number of cases and the lack of biological plausibility raise some doubts between the use of GLP-1 agonists, namely liraglutide, and thyroid cancer occurrence (DRUCKER *et al.*, 2010). Moreover, the effects of this drug in humans, particularly in the human thyroid gland, are unknown and difficult to be extrapolated from pre-clinical studies, despite the C-cell hyperplasia in rats (PAROLA, 2009). The findings of this meta-analysis enhance the need for long-term well-designed epidemiological studies devoted to assess the risk for cancer associated with GLP-1 agonists, including thyroid cancer during liraglutide exposure. Additional studies in animals and the establishment of a cancer registry database to monitor the incidence of medullary thyroid cancer associated with liraglutide was required by the FDA (PARKS and ROSENBRAUGH, 2010).

Some limitations should be considered in this meta-analysis. Few observational longitudinal studies evaluated these AEs. Although three cohort studies have been identified in literature, none observational study evaluating the risk of cancer was found. Since the follow-up time to properly assess these outcomes may take some years, the results of additional studies are expected in the near future. Of the 22 RCTs included, only one considered pancreatitis as an initial outcome measure. Despite two RCTa have evaluated the calcitonin levels, none of them were designed to prospectively monitor for malignancies. The absence of malignancies and/or pancreatitis as pre-defined diagnostic criteria can lead to missing events. Different controls were identified in the RCTs included in this meta-analysis and they might be associated with different risks for acute pancreatitis or cancer, such the case of gliptins or pioglitazone. Because of the heterogeneity of comparators and the relatively small number of acute pancreatitis and cancer events reported in the studies, the stratification of the results at this level is difficult. According to its EPAR, several neoplasms occurred in patients receiving treatment with exenatide BID during the clinical development programme, although the type of carcinomas has not been specified (EUROPEAN MEDICINES AGENCY, 2006). This suggests that publication bias may be present in our meta-analysis despites non-significant results observed for this outcome in the Egger's regression asymmetry test.

The significant between-studies heterogeneity observed for some comparisons established during the meta-analysis evaluating the risk for acute pancreatitis results from the inclusion of observational studies. Therefore, additional caution is needed when interpreting

such risk estimate results. Heterogeneity was considered low when only data from RCTs was included, as was the case of meta-analysis evaluating the risk of cancer or the previous meta-analysis evaluating the safety of coagulation factor Xa direct inhibitors. Nonetheless, due to the very low incidence of carcinomas, the final risk estimates confidence intervals for risk of cancer associated with GLP-1 agonists were found to be wider than those pooled when evaluating the safety of both rivaroxaban and apixaban.

Current available published evidence is insufficient to support an increased risk of acute pancreatitis or an increased risk of cancer from all causes associated with GLP-1 agonists. Since trials' size, duration and design may not be appropriate to accurately assess the risk of rare or long-term AEs, such acute pancreatitis or cancer, clinicians should rely on observational studies in future assessment of the risk of cancer. A rigorous monitoring of these outcomes should be implemented in the future studies since current evidence was not adequately designed to address this issue, precluding any definitive conclusion.

The contribution of meta-analysis for drug safety assessment could be measured through the extent in which regulatory authorities use this tool to support benefit/risk reevaluations. Therefore, a review was conducted in order to assess the type and publication status of data sources supporting benefit/risk ratio reevaluations conducted by four major regulatory authorities on safety issues evaluated between January 2010 and December 2012. The results of this study provide evidence that in the cases of the safety alerts assessed herein regulatory authorities reviewed, either isolated or in combination, several sources of information to support their decisions on safety issues. Such sources of information mainly comprised post-marketing spontaneous reports and experimental and observational clinical studies.

Spontaneously notified reports of cases were present in the majority of the benefit/risk ratio reassessments, with a considerable proportion (20%) of safety evaluations conducted exclusively on this source of evidence. Rare, severe and unexpected AEs, with an acute onset, are prone to be reported by healthcare professionals or patients when they occur within a relatively short period of time after the initiation of the treatment or following a dose increment (MADRE *et al.*, 2006). The value of the pharmacovigilance spontaneous reporting systems in providing evidence on iatrogenic risk is recognized (WYSOWSKI and SWARTZ, 2005; MOORE, SINGH and FURBERG, 2012; LESTER *et al.*, 2013). Reports of cases may be the only available evidence suggesting an association between a suspected drug and an AE. The warnings added to GLP-1 agonists label by FDA regarding the

risk of acute pancreatitis were based on spontaneously reported AEs. The results of experimental and observational studies did not verify such increase in the risk, as well as the results of the meta-analysis conducted on that subject (EUROPEAN MEDICINES AGENCY, 2006; DORE, SEEGER and CHAN, 2009; GARG, CHEN and PENDERGRASS, 2010; DORE *et al.*, 2011).

Evidence from RCTs was found to support a significant number of regulatory decisions. For safety alerts where RCTs were found to be the only data sources consulted, it was not uncommon that the AE being evaluated was an end-point of interest of the study (e.g. QT interval prolongation, bleeding). In other way, several regulatory decisions on AEs occurring with a long-latency time from the initiation of the treatment, such as fractures, cardiovascular events or malignancies, were found to be based on observational designs, the majority being cohort and case-control studies. Although a considerable proportion of the safety alerts was supported by RCTs (41%), this study found that observational data also made a relevant contribution towards supporting safety issues, not only in post-marketing reports of cases (56%) but also in longitudinal studies [cohort (27 %) case-control (22 %)]. Since only the most consumed classes of medicines were included in this work, the probability of these drugs being the subject of observational research is high.

The results of meta-analyses have also been consulted by regulatory authorities to support their decisions, although not so frequently as other data sources. These were meta-analyses conducted using data from RCTs or from observational studies; none pooled data from both experimental and observational studies. When meta-analyses were evaluated, their results were consulted along with those of longitudinal comparative studies. None regulatory authority took decisions based exclusively on the results of meta-analyses. Meta-analysis is not frequently used to support regulatory decisions and, when it does, it is used mainly as a method to confirm the results found in longitudinal comparative studies, whether experimental or observational.

However, a number of limitations should be considered regarding these findings. Regulatory agencies other than FDA, Health Canada, EMA and Australian TGA were not searched and only websites posted in languages other than English were not considered, which may lead to the exclusion of relevant information. Additionally, information on data sources and regulatory actions may not be published in their entirety.

The meta-analytic technique has demonstrated its usefulness in evaluating safety issues. Cumulative meta-analysis has shown that appropriate and timely decisions could have been taken concerning cardiovascular events associated with rofecoxib (US FOOD AND DRUG ADMINISTRATION; 2011). In order to explore if meta-analytic technique would produce

reliable safety risk estimates that could lead regulatory authorities acting earlier than they did, we conducted an additional study. From the previous sample of safety alerts, only those in which regulatory authorities' decisions were supported by longitudinal, comparative studies were selected. The aim of this study was evaluating how risk estimates generated from cumulative meta-analysis performs over time for drugs having their benefit/risk ratio reevaluated and, additionally, assess if the results are consistent with regulatory authorities' conclusions.

The findings of this study show that, for the majority of the safety alerts subject of cumulative meta-analysis (7/9), the risk estimates were in line with the conclusions of the regulatory authorities. Moreover, cumulative risk estimates pointed out that warnings could have been added to the label of proton pump inhibitors in 2004 for Clostridium difficile associated diarrhea and in 2008 for bone fractures. It should be noticed that proton pump inhibitors' labels were subject of first updates in 2012 and 2010 regarding those AEs, respectively. Increased blood sugar was associated with statins in 2008 after pooling data from RCTs. The inclusion of a cohort study in the estimate returned a final result which is statistically non-significant and associated with considerable heterogeneity. Statins' label was updated to properly advise users for the risk of diabetes.

Nonetheless, caution is needed before taking conclusions from these results since they could be somehow biased due to the different study designs included (KIM and BERLIN, 2006; BERLIN, CEPEDA and KIM, 2012). A sensitivity analysis was conducted where the results were pooled according to different study designs, experimental and observational. Meta-analyses exclusively integrating data from RCTs were characterized by low between-studies heterogeneity. Frequent and acute AEs are regularly identified during RCTs, in particular when they are pre-established endpoints of interest. When regulatory authorities and investigators are dealing with rare AEs which may be present in experimental studies, it is frequent to pool data using meta-analysis. This was the case when AEs as cancer and increased blood sugar were evaluated (SATTAR *et al.*, 2010; SIPAHI *et al.*, 2010; VANDENBROUCKE and PSATY, 2008).

All the safety issues studied in this work were evaluated by at least one type of observational methodology when regulatory authorities reviewed their benefit/risk ratio. This could be due to the fact that most of these AEs being considered as rare and/or long-latency events, such as malignancies, cardiovascular events or diabetes, which are prone to be better evaluated in post-authorization safety studies. These studies offer the advantage of a naturalistic observation, which may better represent the incidence of iatrogenic events

occurred in the clinical practice (PAPANIKOLAOU, CHRISTIDI and IOANNIDIS, 2006; ALVES, BATEL-MARQUES and MACEDO, 2012).

Authorities' final conclusions of benefit/risk reevaluations may, in some cases, contradict meta-analytic' risk estimates. Additional data sources supporting a causal relation between an AE and a drug can be used to substantiate regulatory decisions. In such cases, meta-analyses of the existing evidence can return inconclusive results, as it was previously studied for GLP-1 receptor agonists which had labeling updates based on spontaneously reported cases of acute pancreatitis (EOM *et al.*, 2011). In this study, an increased risk of pneumonia associated with proton pump inhibitors was estimated in 2009. This is in line with the results of previous meta-analyses which yielded increased risk estimates and were subsequently reviewed by EMA (GIULIANIO, WILHELM and KALE-PRADHAN, 2012; JOHNSTONE, NERENBERG and LOEB, 2010; EUROPEAN MEDICINES AGENCY, 2012). However, EMA recommended that no risk minimization activities should be taken and kept this class under review. The authority considered that evidence from observational studies of an association between proton pump inhibitors as a class and pneumonia was inconsistent and might be subject to residual confounding (JICK and HERNANDEZ, 2011). Methodological differences between recent studies and those conducted years ago is one of the reasons that may delay the identification of an increased risk, as it was for VTE associated with oral contraceptives containing drospirenone. Differences between VTE definition, risk factors associated with patients and the type of contraceptives previously used in control groups were pointed out as the reasons why the first two studies published in 2007 reported a null association (LESTER *et al.*, 2013). These methodological and clinical issues were taken into consideration in later studies reporting increased risks (LESTER *et al.*, 2013). This may help to explain why only in 2011 an increased risk was pooled by cumulative meta-analysis, the same year that all regulatory authorities suggested labels' updates.

Taking into account the safety issues evaluated in this study and the correspondent regulatory decisions, it is not possible to draw definitive recommendations about the requirements of conducting meta-analyses every time safety signals are issued from data of longitudinal, comparative studies. Observational studies are more susceptible to bias and confounding and integrating data from such designs in meta-analyses may return results with marked heterogeneity across studies, as it was observed for most of the cases evaluated. A sensitivity analysis by study design was conducted to explore its effect as potential source of heterogeneity, but the results did not differ significantly, particularly in those meta-analyses integrating data exclusively from observational studies where higher values of between-

studies heterogeneity obtained in the primary analysis have persisted. When there is little heterogeneity of effects across studies, one may be willing to accept meta-analytic evidence as helping to establish a benefit/risk ratio (KIM and BERLIN, 2006; BERLIN, CEPEDA and KIM, 2012). In the presence of substantial heterogeneity, however, it is difficult to draw definitive conclusions and the acceptance of the results may take time. This might be one of the reasons why regulatory authorities can take several years to conclude on an increased risk in some cases addressed in this study.

This work is subject of some limitations. No systematic bibliographic search was conducted and cumulative meta-analyses were based exclusively on studies used to support safety alerts. Additionally, some of the data sources reviewed by regulatory authorities may not be published on their websites. Although Egger's asymmetry test and visual inspection of funnel plots may not indicate the presence of publication bias in most of the cases, such evaluation is difficult since no specific bibliographic searches were conducted.

The role of meta-analysis in pharmacovigilance is a matter of ongoing debate (DRUG INFORMATION ASSOCIATION, 2011). The Safety Planning, Evaluation and Reporting Team formed in 2006 recommends sponsors to develop a program safety analysis plan beginning with first clinical studies, as a tool to proactively plan for meta-analyses at regular intervals during marketed use of a product (CROWE *et al.*, 2009). The ICH E9 guideline also states that meta-analyses should be prospectively planned with the RCTs program in the development of a new treatment (INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE, 1998).

Council for International Organizations of Medical Sciences (CIOMS) Working Group X was established aiming at developing a consensus on scientific and methodological criteria that represents good practices when applied to meta-analyses of clinical data within the regulatory process (COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES, 2013). These criteria are being developed to be used by both industry and regulators. The working group also intends to develop guidance on how to combine available information from both RCTs and observational studies to generate an integrated result, which is considered controversial. The results should be published in 2014.

The conclusions of the ongoing and further researches should be considered into guidelines where recommendations on how better to combine results from different studies designs in meta-analyses of AEs. Although there exist recommendations on how to conduct

systematic reviews and meta-analyses, none is dedicated to specifically guide researchers on how to integrate different data sources to better assess iatrogenics of interventions.

This work has a number of limitations. When evaluating the methodological differences between different study designs, it is important to consider any confounding factors that may account for any differences identified (GOLDER, LOKE and BLAND, 2011). The assessment of differences between study designs was not a primary aim of this work, but rather to evaluate the influence of different study designs in meta-analytic estimates.

Another limitation is the possibility of underrepresentation of the examples used to explore the initial investigational question. At the time of this researches were conducted, the safety of both GLP-1 receptor agonists and Xa coagulation factor direct inhibitors had been subject of investigation. Meta-analyses of AEs associated with drugs other than those evaluated in this thesis could have resulted in different conclusions.

All the meta-analyses conducted within this thesis have relied on data reported by investigators in the published studies. There was not any attempt to contact the researchers authoring the papers included in the meta-analyses since few hundreds of contacts would have to be established. This was a limitation of similar works (GOLDER, LOKE and BLAND, 2011).

There are a number of methodological considerations that need to be taken into account to design and conduct a meta-analysis. The uncertainty is higher when data from observational studies is integrated, in particular when combined with RCTs. This may be the principal reason for the slowing acceptance of meta-analysis as a tool in the medicines' benefit/risk ratio reevaluations following safety signals. Between-studies heterogeneity due to clinical and/or methodological differences may delay the conclusions of the decision-making process even when meta-analytic' pooled estimates found an increase in risk. There is a need to explore the between-studies heterogeneity from two perspectives, clinical and methodological. Clinical heterogeneity refers to differences associated with the participants, interventions or outcomes. The participants may differ for example in age or gender, the interventions may differ in type, dose and duration; and the definitions of the outcomes measured may differ, as well as the duration of follow-up. Methodological heterogeneity refers to differences in the way the studies were conducted, for example, differences in study design or risk of bias. Even though a review deliberately selects studies that may be

similar in many ways based on these factors, there can still be substantial differences that mean it might not make sense to pool their results.

The results of this work suggests that instead of restricting meta-analyses to one type of study design, a broad range of studies should be searched and considered for inclusion in the pooled estimates. Previous research recommends that systematic review of literature should not be restricted to specific study types and that both experimental and observational data should be included in meta-analyses of AEs of pharmacological interventions (GOLDER, LOKE and BLAND, 2011). Nonetheless, since the results of meta-analyses including different types of studies can be associated with higher uncertainty, further risk assessments based on the results from other data sources should be considered in the decision-making process.

VII.2. BIBLIOGRAPHY

ALVES, C.; BATEL-MARQUES, F.; MACEDO, A.F. – A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. **Diabetes Res Clin Pract.** 98;2 (2012) 271-284.

BALANI, A.R.; GRENDELL, J.H. – Drug-induced pancreatitis: incidence, management, and prevention. **Drug Saf**. 31;10 (2008) 823-837.

BECATTINI, C.; LIGNANI, A.; AGNELLI, G. – New oral anticoagulants for the prevention of venous thromboembolism. **Drug Des Devel Ther.** 4 (2010) 49-60.

BJERRE KNUDSEN, L. [et al] – Glucagon-like peptide-1 receptor agonists active rodent thyroid C-cells causing calcitonin release and C-cell proliferation. **Endocrinology**. 151;4 (2010) 1473-1486.

CAO, Y.B. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after total hip or knee arthroplasty: a meta-analysis of randomized controlled trials. **Eur J Clin Pharmacol.** 66;11 (2010) 1099-1108.

CHEN, L.C.; ASHCROFT, D.M. – Do selective COX-2 inhibitors increase the risk of cerebrovascular events? A meta-analysis of randomized controlled trials. **J Clin Pharm Ther.** 31 (2006) 565-576.

COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES – CIOMS Working Group X on "Considerations for applying good meta-analysis practices to clinical safety data within the biopharmaceutical regulatory process". (2013) Available from: http://www.cioms.ch/index.php/2012-06-10-08-47-53/working-groups/working-group-x

CROWE, B.J. [et al] – Recommendations for safety planning, data collection, evaluation and reporting during drug, biologic and vaccine development: a report of the safety planning, evaluation, and reporting team. **Clin Trials.** 6;5 (2009) 430-440.

DORE, D.D. [et al] – A cohort study of acute pancreatitis in relation to exenatide use. **Diabetes Obes Metab.** 13;6 (2011) 559-566.

DORE, D.D.; SEEGER, J.D.; CHAN, K.A. – Use of a claims-based active drug safety surveillance system to assess the risk of acute pancreatitis with exenatide or sitagliptin compared to metformin or glyburide. **Curr Med Res Opin.** 25;4 (2009) 1019-1027.

DOWNS, S.H.; BLACK, N. – The feasibility of creating a checklist for assessment of the methodological quality of both the randomised and nonrandomised studies of health care interventions. **J Epidemiol Community Health.** 52;6 (1998) 377-384.

DRUCKER, D.J. [et al] – Increatin-based therapies for the treatment of type 2 diabetes: evaluation of the risks and benefits. **Diabetes Care**. 33;2 (2010) 428-433.

DRUG INFORMATION ASSOCIATION – DIA/FDA Best practices for regulatory information synthesis of randomized controlled trials for product safety evaluation. Drug Information Association, Inc. (2011) Available from http://www.diahome.org/DIAHome/resources/content.aspx?type=eopdf&file=%2fproductfiles%2f25159%2f11011%2Epdf.

EGGER, M.; SCHNEIDER, M.; DAVEY SMITH, G. – Spurious precision? Meta-analysis of observational studies. **BMJ**. 316;7125 (1998) 140-144.

EGGER, M.; SMITH, G.D.; PHILLIPS, A.N. - Meta-analysis: principles and procedures. **BMJ**. 315;7121 (1997) 1533-1537.

ELASHOFF, M. [et al] – Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. **Gastroenterology**. 141;1 (2011) 150-156.

EOM, C.S. [et al] – Use of acidsuppressive drugs and risk of pneumonia: a systematic review and meta-analysis. **Can Med Assoc J.** 183;3 (2011) 310-319.

ERIKSSON, B.I. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. **N Engl J Med.** 358;26 (2008) 2765-2775.

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Bydureon. Committee for Medicinal Products for Human Use (CHMP). (2011) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002020/human_med_001457.jsp&mid=WC0b01ac058001d124.

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Byetta. Committee for Medicinal Products for Human Use (CHMP). (2006) Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000698/human_med_000682.jsp&mid=WC0b01ac058001d124.

EUROPEAN MEDICINES AGENCY – CHMP assessment report for Victoza. EMEA/379172/2009. Committee for Medicinal Products for Human Use (CHMP). (2009) Available from:

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/001026/human_med_001137.jsp&mid=WC0b01ac058001d124.

EUROPEAN MEDICINES AGENCY – PhVWP monthly report on safety concerns, guidelines and general matters July 2012 – Issue number: 1207. EMA/CHMP/PhVWP/438980/2012. Committee for Medicinal Products for Human Use (CHMP). (2012) Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2012/07/WC500130391.pdf

GAGNE, J.J.; GRIESDALE, D.E.G.; SCHNEEWEISS, S. – Aprotin and the risk of death and renal dysfunction in patients undergoing cardiac surgery: a meta-analysis of epidemiologic studies. **Pharmacoepidemiol Drug Saf.** 2009; 18: 259-268.

GARG, R.; CHEN, W.; PENDERGRASS, M. – Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. **Diabetes Care.** 33;11 (2010) 2349-2354.

GEERTS, W.H. [et al] – Prevention of venous thromboembolism: American college of chest physicians evidence-based clinical practice guidelines. 8th ed. **Chest**. 133;6 suppl (2008) 381S-453S.

GIULIANIO, C.; WILHELM, S.M.; KALE-PRADHAN, P.B. – Are proton pump inhibitors associated with the development of community-acquired pneumonia?: a meta-analysis. **Expert Rev Clin Pharmacol.** 5;3 (2012) 337-344.

GOLDER, S.; LOKE, Y.K.; BLAND, M. – Meta-analyses of adverse effects data derived from randomised controlled trials as compared to observational studies: methodological overview. **PLoS Med.** 8;5 (2011) e1001026. doi: 10.1371/journal.pmed.1001026.

GÓMEZ-OUTES, A. [et al] – Rivaroxaban versus enoxaparin after total knee arthroplasty. **Lancet.** 374;9691 (2009) 682.

GROSS, P.L.; WEITZ, J.I. – Newanticoagulants for treatment of venous thromboembolism. **Arterioscler Throm Vasc Biol.** 28;3 (2008) 380-386.

HAUSMANN, O.; SCHNYDER, B.; PICHLER, W.J. – Etiology and pathogenesis of adverse drug reactions. **Chem Immunol Allergy.** 97 (2012) 32-46.

HENNESSY, S. [et al] – Risk of venous thromboembolism form oral contraceptives containing gestodene and desogestrel versus levonogestrel: a meta-analysis and formal sensitivity analysis. **Contraception.** 2001; 64: 125-133.

HOPEWELL, S.; WOLFENDEN, L.; CLARKE, M. – Reporting of adverse events in systematic reviews can be improved: survey results. **J Clin Epidemiol** 61;6 (2008) 597-602.

HUANG, J. [et al] – Apixaban versus enoxaparin in patients with total knee arthroplasty: a meta-analysis of randomised trials. **Thromb Haemost.** 105;2 (2011) 245-253.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE – Statistical principles for clinical trials: E9, 1998.

IOANNIDIS, J.P. – Adverse events in randomized trials: neglected, restricted, distorted, and silenced. **Arch Intern Med.** 169;19 (2009) 1737-1739.

JENSEN, C.D. [et al] – Return to theatre following total hip and knee replacement, before and after introduction of rivaroxaban: a retrospective cohort study. **J Bone Joint Surg Br.** 93;1 (2011) 91-95.

JOHNSTONE, J.; NERENBERG, K.; LOEB, M. – Meta-analysis: proton pump inhibitor use and the risk of community-acquired pneumonia. **Aliment Pharmacol Ther.** 31;11 (2010) 1165-1177.

JÜNI, P. [et al] – The hazards of scoring the quality of clinical trials for meta-analysis. **JAMA**. 282;11 (1999) 1054-1060.

KIM, C.J.; BERLIN, J.A. – The use of meta-analysis in pharmacoepidemiology**.** In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 353-366.

LASSEN, M.R. [et al] – Apixaban or enoxaparin for thromboprophylaxis after knee replacement. **N Engl J Med.** 361;6 (2009) 594-604.

LASSEN, M.R. [et al] – Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. **Lancet.** 375;9717 (2010) 807-815.

LASSER, K.E. [et al] – Timing of new black box warnings and withdrawals for prescription medications. **JAMA.** 287;17 (2002) 2215-2220.

LESTER, J. [et al] – Evaluation of FDA safety-related drug label changes in 2010. **Pharmacoepidemiol Drug Saf.** 22;3 (2013) 302-305.

LIDEGAARD, Ø. [et al] – Hormonal contraception and risk of venous thromboembolism: national follow-up study. **BMJ**. 339 (2009) b2890.

LOKE, Y.K.; JEEVANANTHAM, V.; SINGH, S. – Biphosphonates and atrial fibrillation: systematic review and meta-analysis. **Drug Saf.** 32;3 (2009) 219-228.

LOTKE, P.A. – Rivaroxaban for thromboprophylaxis. **N Engl J Med.** 359;20 (2008) 2174.

MADRE, L.K. [et al] – Views from Academia, Industry, and Regulatory Agencies. In: STROM, Brian; KIMMEL, Stephen, Textbook of pharmacoepidemiology. 4th Ed., Chichester, England: John Wiley & Sons, 2006, ISBN: 978-0-470-02925-1. p. 63-88.

MOORE, T.J.; SINGH, S.; FURBERG, C.D. – The FDA and new safety warnings. **Arch Intern Med.** 172;1 (2012) 78-80.

NATHAN, D.M. [et al] – Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. **Diabetes Care.** 32;1 (2009) 193-203.

NOEL, R.A. [et al] – Increased risk of acute pancreatitis and biliary disease observed in patients with type 2 diabetes. **Diabetes Care.** 32;5 (2009) 834-838.

PAPANIKOLAOU, P.N.; CHRISTIDI, G.D.; IOANNIDIS, J.P.A. – Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. **CMAJ**. 174;5 (2006) 635-641.

PARKS, M.; ROSENBRAUGH, C. – Weighting risks and benefits of liraglutide - the FDA's review of a new antidiabetic therapy. **N Engl J Med.** 362;9 (2010) 774-777.

PAROLA, T. – Us Food And Drug Administration. Victoza® (liraglutide injection): human relevance of rodent thyroid C-cell tumors. Centre for Drug Evaluation and Research. (2009) Available from: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM151129.pdf

PLATT, R. [et al] – Observational studies of drug safety in distributed data networks: experience from the Canadian Network for Observational Drug Effect Studies. (2014)Available from: http://www.ihdlnconference2014.org/subpanel/Platt.

RASCHI, E. [et al] – The association of pancreatitis with antidiabetic drug use: gaining insight through the FDA pharmacovigilance database. **Acta Diabetol.** 50;4 (2013) 569-577.

SATTAR, N. [et al] – Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. **Lancet.** 375;9716 (2010) 735-742.

SIPAHI, I. [et al] – Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. **Lancet Oncol.** 11;7 (2010) 627-636.

STAFFA, J.A.; CHANG, J.; GREEN, L. – Cerivastatin and reports of fatal rhabdomyolysis. **N Engl J Med.** 346;7 (2002) 539-540.

TOH, S.; HERNÁNDEZ-DÍAZ, S. – Statins and fracture risk: a systematic review. **Pharmacoepidemiol Drug Saf.** 2007; 16: 627-640.

TURPIE, A.G. [et al] – Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. **Lancet.** 373;9676 (2009) 1673-1680.

US FOOD AND DRUG ADMINISTRATION – Byetta (exenatide) injection. Detailed view: safety labelling changes approved by FDA Centre for Drug Evaluation and Research. MedWatch the FDA Safety Information and Adverse Event Reporting Program. (2009) Available from: http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm190571.htm

US FOOD AND DRUG ADMINISTRATION – Guidance for Industry – Warnings and precautions, contraindications, and boxed warning sections of labeling for human prescription drug and biological products – content and format. Centre for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). (2011) Available from: http://www.fda.gov/downloads/Drugs/Guidances/ucm075096.pdf.

US FOOD AND DRUG ADMINISTRATION – Information for Healthcare Professionals: Exenatide (marketed as Byetta) – 10/2007. Postmarket Drug Safety Information for Patients and Providers. (2007) Available from: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm124712.htm.

US FOOD AND DRUG ADMINISTRATION – VICTOZA DRUG APPROVAL PACKAGE. CENTRE FOR DRUG EVALUATION AND RESEARCH. (2010) AVAILABLE FROM: HTTP://WWW.ACCESSDATA.FDA.GOV/DRUGSATFDA_DOCS/NDA/2010/022341S000TOC.CFM

VANDENBROUCKE, J.P. – What is the best evidence for determining harms of medical treatment? **CMAJ** 174;5 (2006) 645-646.

VANDENBROUCKE, J.P. – When are observational studies as credible as randomised trials? **Lancet**. 363;9422 (2004) 1728-1731.

VANDENBROUCKE, J.P.; PSATY, B.C. – Benefits and risks of drug treatments: how combine the best evidence on benefits with the best data about adverse effects. **JAMA**. 300;20 (2008) 2417-2419.

WEITZ, J.I. – New oral anticoagulants in development. **Thromb Haemost.** 103;1 (2010) 62-70.

WYSOWSKI, D.K.; SWARTZ, L. – Adverse drug event surveillance and drug withdrawals in the United States, 1969-2002: the importance of reporting suspected reactions. **Arch Intern Med.** 165;12 (2005) 1363-1369.

BERLIN, J.; SOLEDAD CEPEDA, M.; KIM, C. – **The Use of Meta-analysis in Pharmacoepidemiology** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 723-756.

LESKO, S.; MITCHELL, A. – **The Use of Randomized Controlled Trials for Pharmacoepidemiologic Studies.** In: STROM, Brian; KIMMEL, Stephen, HENNESSY, Sean, Pharmacoepidemiology. 5th Ed., Chichester, England: John Wiley & Sons, 2012, ISBN: 978-0-470-65475-0. p. 640-654.

CHAPTER VIII – FINAL CONCLUSIONS

VIII. FINAL CONCLUSIONS

VIII.1. CONCLUSIONS

This thesis evaluated the potential usefulness of meta-analysis for Pharmacovigilance, exploring if this statistical technique would produce reliable estimates when combining results from different data sources, namely experimental and observational studies. In order to answer to the initial research questions, several studies were conducted. Briefly, the most relevant conclusions obtained throughout the work developed under this thesis are the following:

- The majority of meta-analyses published by the highly impact medical journals are designed to assess the efficacy of pharmacological interventions; only a limited number of meta-analyses are currently devoted to evaluate drug safety as a primary outcome. Randomized clinical trials are the main source of information from where data is pooled off; very few meta-analyses included data from both observational and experimental studies. Although meta-analyses including observational studies could be more frequently published in journals devoted to the pharmacoepidemiology and pharmacovigilance areas, the most read and quoted medical journals are those which will influence the clinical practice and prompt regulatory authorities to act.

- The results of meta-analyses of RCTs are less affected by between-studies heterogeneity, in particular when such trials have similar methodological design. The risk estimates for bleedings associated with Xa coagulation factor direct inhibitors, rivaroxaban and apixaban, were characterized by lower between-studies heterogeneity. The same was observed in the meta-analysis evaluating the risk of cancer associated with GLP-1 agonists, exenatide and liraglutide, although the rarity of this particular event have produced wider confidence intervals. In this particular case, the divergence between the risk of cancer associated with exenatide and liraglutide which was identified in the meta-analysis demonstrates that this technique may be useful in generating research hypothesis and, lately, safety signals.

- The inclusion of observational studies in meta-analysis, isolated or in combination with RCTs, leads to an increase in the between-studies heterogeneity. The risk estimates of such meta-analyses can produce statistical significant results, but should be interpret with caution due to the uncertainty produced by data pooled from different study designs. Pooled estimates showed no increased risk of acute pancreatitis associated with GLP-1 agonists. However, the inclusion of observational studies resulted in higher between-studies heterogeneity.

- Spontaneously reported suspected adverse drug reaction support most of the post-marketing benefit/risk ratio reevaluations conducted by regulatory authorities. It was identified that post-market safety regulatory decisions could be supported by meta-analyses' results, although less frequently. None regulatory decision used meta-analyses combining both experimental and observational studies as well as none authority used meta-analysis in exclusive when decided to act upon a safety issued.

- For safety alerts based on data from longitudinal, comparative studies, namely RCTs and observational studies (cohort and case-control), meta-analysis was able to produce risk estimates in line with authorities' conclusions in the majority of the situations. It was also demonstrated that cumulative meta-analysis was able to predict iatrogenic risks earlier than authorities' regulatory decisions. However, when there is a need to integrate data from both experimental and observational studies, results can be affected by excessive heterogeneity. This may delay regulatory authorities to accept the results of meta-analyses combining data from different study designs.

Although reliable risk estimates have shown to be produced from meta-analyses conducted to evaluate drug safety issues, between-studies heterogeneity may preclude investigators and regulatory authorities from draw robust conclusions from those results. Uncertainty may increase when observational data is pooled, in exclusive or in combination with experimental studies. The findings of this work do not let to recommend that a meta-analysis of the existing evidence should be conducted whenever a drug-safety alert is issued.

Moreover, this technique does not replace further assessments when the benefit/risk ratio profile of a medicine needs to be revised due to an increased risk of a suspected ADR.